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(54) Title: INHIBITORS OF PRENYL-PROTEIN TRANSFERASE

(57) Abstract: The present invention is directed to piperidine- and pyrrolidine-containing macrocyclic compounds which inhibit prenyl-protein transferase and the prenylation of the oncogene protein Ras. The invention is further directed to chemotherapeutic compositions containing the compounds of this invention and methods for inhibiting prenyl-protein transferase and the prenylation of the oncogene protein Ras.



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TITLE OF THE INVENTION

INHIBITORS OF PRENYL-PROTEIN TRANSFERASE

BACKGROUND OF THE INVENTION

The Ras proteins (Ha-Ras, Ki4a-Ras, Ki4b-Ras and N-Ras) are part of a signaling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Biological and biochemical studies of Ras action indicate that Ras functions like a G-regulatory protein. In the inactive state, Ras is bound to GDP. Upon growth factor receptor activation Ras is induced to exchange GDP for GTP and undergoes a conformational change. The GTP-bound form of Ras propagates the growth stimulatory signal until the signal is terminated by the intrinsic GTPase activity of Ras, which returns the protein to its inactive GDP bound form (D.R. Lowy and D.M. Willumsen, *Ann. Rev. Biochem.* 62:851-891 (1993)). Mutated *ras* genes (Ha-*ras*, Ki4a-*ras*, Ki4b-*ras* and N-*ras*) are found in many human cancers including colorectal carcinoma, exocrine pancreatic carcinoma, and myeloid leukemias. The protein products of these genes are defective in their GTPase activity and constitutively transmit a growth stimulatory signal.

Ras must be localized to the plasma membrane for both normal and oncogenic functions. At least 3 post-translational modifications are involved with Ras membrane localization, and all 3 modifications occur at the C-terminus of Ras. The Ras C-terminus contains a sequence motif termed a "CAAX" or "Cys-Aaa¹-Aaa²-Xaa" box (Cys is cysteine, Aaa is an aliphatic amino acid, the Xaa is any amino acid) (Willumsen et al., *Nature* 310:583-586 (1984)). Depending on the specific sequence, this motif serves as a signal sequence for the enzymes farnesyl-protein transferase or geranylgeranyl-protein transferase type I, which catalyze the alkylation of the cysteine residue of the CAAX motif with a C₁₅ or C₂₀ isoprenoid, respectively. (S. Clarke., *Ann. Rev. Biochem.* 61:355-386 (1992); W.R. Schafer and J. Rine, *Ann. Rev. Genetics* 30:209-237 (1992)). The term prenyl-protein transferase may be used to refer generally to farnesyl-protein transferase and geranylgeranyl-protein transferase type I. The Ras protein is one of several proteins that are known to undergo post-translational farnesylation. Other farnesylated proteins include the Ras-related GTP-binding proteins such as Rho, fungal mating factors, the nuclear lamins, and the gamma subunit of transducin. James, et al., *J. Biol. Chem.* 269, 14182 (1994) have identified a peroxisome associated protein Pxf which is also farnesylated. James, et al., have also suggested that there are farnesylated proteins

of unknown structure and function in addition to those listed above.

Inhibition of farnesyl-protein transferase has been shown to block the growth of Ras-transformed cells in soft agar and to modify other aspects of their transformed phenotype. It has also been demonstrated that certain inhibitors of farnesyl-protein transferase selectively block the processing of the Ras oncoprotein intracellularly (N.E. Kohl *et al.*, *Science*, 260:1934-1937 (1993) and G.L. James *et al.*, *Science*, 260:1937-1942 (1993). Recently, it has been shown that an inhibitor of farnesyl-protein transferase blocks the growth of *ras*-dependent tumors in nude mice (N.E. Kohl *et al.*, *Proc. Natl. Acad. Sci U.S.A.*, 91:9141-9145 (1994) and induces regression of mammary and salivary carcinomas in *ras* transgenic mice (N.E. Kohl *et al.*, *Nature Medicine*, 1:792-797 (1995).

Indirect inhibition of farnesyl-protein transferase *in vivo* has been demonstrated with lovastatin (Merck & Co., Rahway, NJ) and compactin (Hancock *et al.*, *ibid*; Casey *et al.*, *ibid*; Schafer *et al.*, *Science* 245:379 (1989)). These drugs inhibit HMG-CoA reductase, the rate limiting enzyme for the production of poly-isoprenoids including farnesyl pyrophosphate. Farnesyl-protein transferase utilizes farnesyl pyrophosphate to covalently modify the Cys thiol group of the Ras CAAX box with a farnesyl group (Reiss *et al.*, *Cell*, 62:81-88 (1990); Schaber *et al.*, *J. Biol. Chem.*, 265:14701-14704 (1990); Schafer *et al.*, *Science*, 249:1133-1139 (1990); Manne *et al.*, *Proc. Natl. Acad. Sci USA*, 87:7541-7545 (1990)). Inhibition of farnesyl pyrophosphate biosynthesis by inhibiting HMG-CoA reductase blocks Ras membrane localization in cultured cells. However, direct inhibition of farnesyl-protein transferase would be more specific and attended by fewer side effects than would occur with the required dose of a general inhibitor of isoprene biosynthesis.

Inhibitors of farnesyl-protein transferase (FPTase) have been described in two general classes. The first are analogs of farnesyl diphosphate (FPP), while the second class of inhibitors is related to the protein substrates (e.g., Ras) for the enzyme. The peptide derived inhibitors that have been described are generally cysteine containing molecules that are related to the CAAX motif that is the signal for protein prenylation. (Schaber *et al.*, *ibid*; Reiss *et al.*, *ibid*; Reiss *et al.*, *PNAS*, 88:732-736 (1991)). Such inhibitors may inhibit protein prenylation while serving as alternate substrates for the farnesyl-protein transferase enzyme, or may be purely competitive inhibitors (U.S. Patent No. 5,141,851, University of Texas; N.E. Kohl *et al.*, *Science*, 260:1934-1937 (1993); Graham, *et al.*, *J. Med. Chem.*, 37, 725 (1994)). In general, deletion of the thiol from a CAAX derivative has been shown

to dramatically reduce the inhibitory potency of the compound. However, the thiol group potentially places limitations on the therapeutic application of FPTase inhibitors with respect to pharmacokinetics, pharmacodynamics and toxicity. Therefore, a functional replacement for the thiol is desirable.

5 It has recently been reported that farnesyl-protein transferase inhibitors are inhibitors of proliferation of vascular smooth muscle cells and are therefore useful in the prevention and therapy of arteriosclerosis and diabetic disturbance of blood vessels (JP H7-112930).

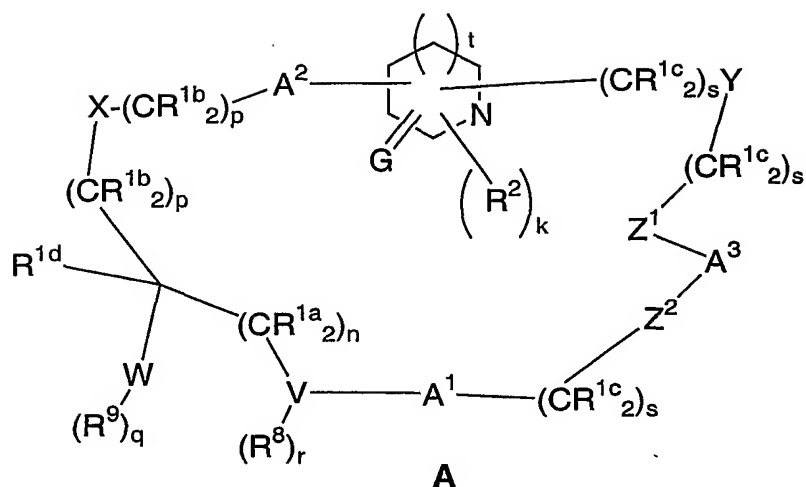
10 It has recently been disclosed that certain tricyclic compounds which optionally incorporate a piperidine moiety are inhibitors of FPTase (WO 95/10514, WO 95/10515 and WO 95/10516). Imidazole-containing inhibitors of farnesyl protein transferase have also been disclosed (WO 95/09001 and EP 0 675 112 A1).

15 It is, therefore, an object of this invention to develop peptidomimetic compounds that do not have a thiol moiety, and that will inhibit prenyl-protein transferase and thus, the post-translational prenylation of proteins. It is a further object of this invention to develop chemotherapeutic compositions containing the compounds of this invention and methods for producing the compounds of this invention.

20 SUMMARY OF THE INVENTION

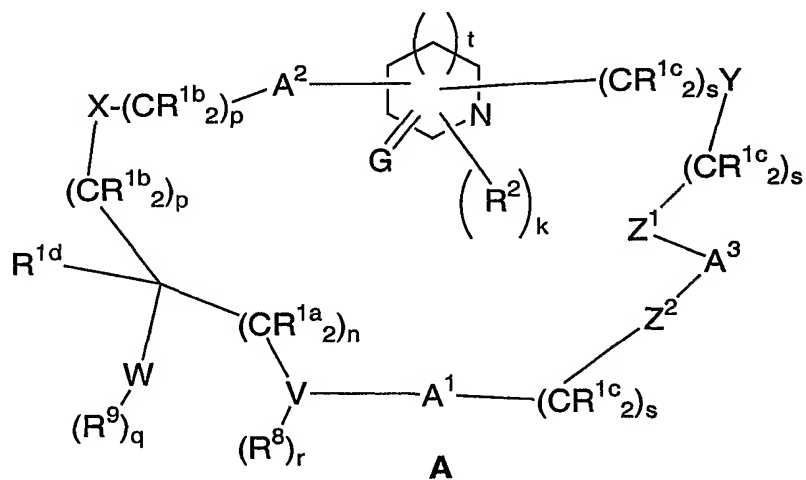
25 The present invention comprises peptidomimetic piperidine- and pyrrolidine-containing macrocyclic compounds which inhibit prenyl-protein transferase. Further contained in this invention are chemotherapeutic compositions containing these prenyl-protein transferase inhibitors and methods for their production.

 The compounds of this invention are illustrated by the formula A:



DETAILED DESCRIPTION OF THE INVENTION

- The compounds of this invention are useful in the inhibition of prenyl-protein transferase and the prenylation of the oncogene protein Ras. In a first embodiment of this invention, the inhibitors of prenyl-protein transferase are illustrated by the formula A:



wherein:

- 10 R^{1a}, R^{1b}, R^{1c} and R^{1d} are independently selected from:
- hydrogen,
 - unsubstituted or substituted aryl,
 - unsubstituted or substituted heterocycle,

- 5
- d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 - e) R¹⁰O-,
 - f) R¹¹S(O)_m-,
 - g) R¹⁰C(O)NR¹⁰-,
 - h) (R¹⁰)₂NC(O)-,
 - i) CN,
 - j) NO₂,
 - k) R¹⁰C(O)-,
 - l) R¹⁰OC(O)-,
 - 10 m) -N(R¹⁰)₂,
 - n) R¹¹OC(O)NR¹⁰-,
 - o) unsubstituted or substituted C₁-C₆ alkyl,
 - p) unsubstituted or substituted C₂-C₆ alkenyl, or
 - q) unsubstituted or substituted C₂-C₆ alkynyl,

15

wherein the substituent on the substituted group is selected from unsubstituted or substituted C₁-C₆ alkyl, unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, unsubstituted or substituted C₃-C₁₀ cycloalkyl, unsubstituted or substituted C₂-C₆ alkenyl, unsubstituted or substituted C₂-C₆ alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, and R¹¹OC(O)-NR¹⁰-,

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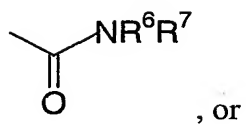
or two R^{1a}s, two R^{1b}s or two R^{1c}s, on the same carbon atom may be combined to form -(CH₂)_u-;

25

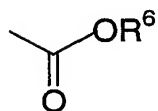
R² is independently selected from

- a) hydrogen,
- b) unsubstituted or substituted C₁₋₈ alkyl,
- c) unsubstituted or substituted C₂₋₈ alkenyl,
- 30 d) unsubstituted or substituted C₂₋₈ alkynyl,
- e) unsubstituted or substituted aryl,
- f) unsubstituted or substituted heterocycle,

g)



h)



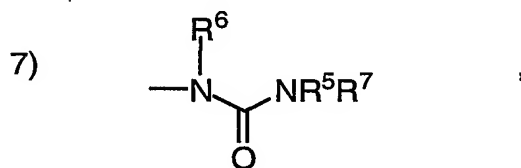
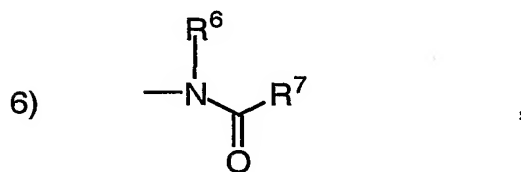
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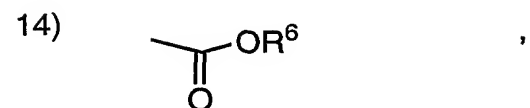
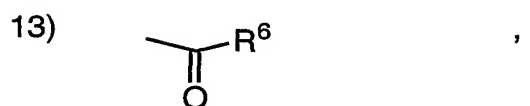
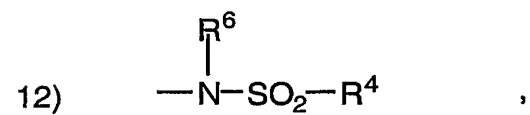
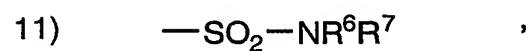
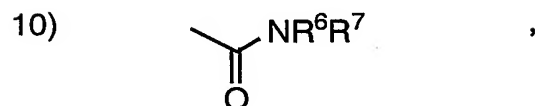
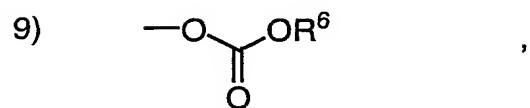
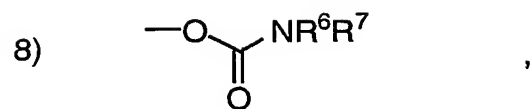
wherein the substituted group is substituted with one or more of:

- 1) aryl or heterocycle, unsubstituted or substituted with:
 - a) C₁₋₄ alkyl,
 - b) (CH₂)_pOR⁶,
 - c) (CH₂)_pNR⁶R⁷,
 - d) halogen,
 - e) CN,
- 2) C₃₋₆ cycloalkyl,
- 3) OR⁶,
- 4) SR⁴, S(O)R⁴, SO₂R⁴,

10

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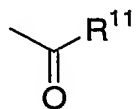




5 R^4 is selected from C_{1-6} alkyl, C_{3-6} cycloalkyl, heterocycle, aryl, unsubstituted or substituted with:

- 1) C_{1-6} alkoxy,
- 2) aryl or heterocycle,
- 3) halogen,
- 4) HO ,
- 5)

10

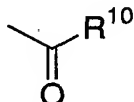


- 6) SO_2R^{11} ,
- 7) $\text{N}(\text{R}^{10})_2$, or
- 8) C_{1-4} perfluoroalkyl;

5 R^5 , R^6 and R^7 are independently selected from:

- a) hydrogen,
 - b) $\text{R}^{10}\text{C}(\text{O})-$,
 - c) $\text{R}^{10}\text{OC}(\text{O})-$, or
 - d) C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl,
- 10 heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with one or more substituents selected from:

- 1) unsubstituted or substituted C_1 - C_6 alkyl,
- 2) $\text{R}^{10}\text{O}-$,
- 15 3) unsubstituted or substituted aryl or heterocycle,
- 4) halogen,
- 5) $\text{R}^{10}\text{C}(\text{O})\text{NR}^{10}-$,
- 6)



20

- 7) SO_2R^{11} ,
- 8) $\text{N}(\text{R}^{10})_2$,
- 9) C_{3-6} cycloalkyl,
- 10) C_{1-6} perfluoroalkyl,
- 25 11) $\text{R}^{10}\text{OC}(\text{O})-$,
- 12) $\text{R}^{11}\text{OC}(\text{O})\text{NR}^{10}-$,
- 13) CN , and
- 14) NO_2 ; or

30 R^6 and R^7 may be joined in a ring; and independently,

R^5 and R^7 may be joined in a ring;

R⁸ is independently selected from:

- a) hydrogen,
- b) unsubstituted or substituted aryl,
- c) unsubstituted or substituted heterocycle,
- 5 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- e) unsubstituted or substituted C₂-C₆ alkenyl,
- f) unsubstituted or substituted C₂-C₆ alkynyl,
- g) C₁-C₆ perfluoroalkyl,
- h) halo,
- 10 i) R¹⁰O-,
- j) R¹¹S(O)_m-,
- k) R¹⁰C(O)NR¹⁰-,
- l) (R¹⁰)₂NC(O)-,
- m) CN,
- 15 n) NO₂,
- o) R¹⁰C(O)-,
- p) R¹⁰OC(O)-,
- q) -N(R¹⁰)₂,
- r) R¹¹OC(O)NR¹⁰-, and
- 20 s) unsubstituted or substituted C₁-C₆ alkyl, optionally substituted by unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, unsubstituted or substituted C₃-C₁₀ cycloalkyl, unsubstituted or substituted C₂-C₆ alkenyl, unsubstituted or substituted C₂-C₆ alkynyl, C₁-C₆ perfluoroalkyl, F, Cl, Br, R¹⁰O-,
- 25 R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹⁰OC(O)NR¹⁰-;

R⁹ is selected from:

- a) hydrogen,
- 30 b) unsubstituted or substituted C₂-C₆ alkenyl,
- c) unsubstituted or substituted C₂-C₆ alkynyl,
- d) C₁-C₆ perfluoroalkyl,
- e) halo,
- f) R¹⁰O-,

- 5 g) $R^{11}S(O)_m-$,
 h) $R^{10}C(O)NR^{10}-$,
 i) $(R^{10})_2NC(O)-$,
 j) CN,
 k) NO_2 ,
 l) $R^{10}C(O)-$,
 m) $R^{10}OC(O)-$,
 n) $-N(R^{10})_2$,
 o) $R^{11}OC(O)NR^{10}-$, or
 10 p) unsubstituted or substituted C_1 - C_6 alkyl optionally substituted by aryl,
 C_3 - C_{10} cycloalkyl, C_1 - C_6 perfluoroalkyl, F, Cl, Br, $R^{10}O-$,
 $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, $(R^{10})_2NC(O)-$, CN, $R^{10}C(O)-$,
 $R^{10}OC(O)-$, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$;

15 R^{10} is independently selected from

- a) hydrogen,
 b) unsubstituted or substituted C_1 - C_6 alkyl,
 c) unsubstituted or substituted C_1 - C_3 perfluoroalkyl,
 d) unsubstituted or substituted benzyl,
 20 e) unsubstituted or substituted aryl, or
 f) unsubstituted or substituted heterocycle;

R^{11} is independently selected from

- a) unsubstituted or substituted C_1 - C_6 alkyl,
 25 b) unsubstituted or substituted aryl, or
 c) unsubstituted or substituted heterocycle;

A^1 and A^3 are independently selected from

- a) a bond,
 30 b) $-C(O)-$,
 c) $-C(O)NR^{10}-$,
 d) $-NR^{10}C(O)-$,
 e) O,
 f) $-N(R^{10})-$,

- g) $-\text{S}(\text{O})_2\text{N}(\text{R}^{10})-$,
- h) $-\text{N}(\text{R}^{10})\text{S}(\text{O})_2-$, or
- i) $\text{S}(\text{O})_m$;

5 A² is selected from

- a) a bond,
- b) $-\text{C}(\text{O})-$,
- c) $-\text{C}(\text{O})\text{NR}^{10}-$,
- d) $-\text{NR}^{10}\text{C}(\text{O})-$,
- 10 e) O,
- f) $-\text{N}(\text{R}^{10})-$,
- g) $-\text{S}(\text{O})_2\text{N}(\text{R}^{10})-$,
- h) $-\text{N}(\text{R}^{10})\text{S}(\text{O})_2-$,
- i) $-\text{NR}^{10}\text{C}(\text{O})\text{NR}^{10}-$, or
- 15 j) $\text{S}(\text{O})_m$;

G is selected from H₂ or O;

V is selected from:

- 20 a) heterocycle, or
- b) aryl;

W is a heterocycle;

25 X is selected from:

- a) a bond,
- b) $-\text{C}(\text{O})-$,
- c) $-\text{C}(\text{O})\text{NR}^{10}-$,
- d) $-\text{NR}^{10}\text{C}(\text{O})-$,
- 30 e) $-\text{NR}^{10}\text{C}(\text{O})-\text{O}-$,
- f) $-\text{O}-\text{C}(\text{O})\text{NR}^{10}-$,
- g) $-\text{NR}^{10}\text{C}(\text{O})\text{NR}^{10}-$,
- h) O,
- i) $-\text{N}(\text{R}^{10})-$,

- j) $-\text{S}(\text{O})_2\text{N}(\text{R}^{10})-$,
- k) $-\text{N}(\text{R}^{10})\text{S}(\text{O})_2-$, or
- l) $\text{S}(\text{O})_m$;

5 Y is selected from

- a) a bond,
- b) $-\text{C}(=\text{O})-$,
- c) $-\text{C}(\text{O})\text{NR}^{10}-$,
- d) $-\text{C}(\text{O})\text{O}-$, or
- 10 e) $-\text{S}(=\text{O})_m$;

Z^1 is selected from unsubstituted or substituted aryl and unsubstituted or substituted heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with one or more of:

- 15 1) C_1 -8 alkyl, C_2 -8 alkenyl or C_2 -8 alkynyl, unsubstituted or substituted with:
 - a) C_1 -4 alkoxy,
 - b) NR^6R^7 ,
 - c) C_3 -6 cycloalkyl,
 - 20 d) aryl or heterocycle,
 - e) HO ,
 - f) $-\text{S}(\text{O})_m\text{R}^4$,
 - g) $-\text{C}(\text{O})\text{NR}^6\text{R}^7$, or
 - h) C_1 -4 perfluoroalkyl;
- 25 2) substituted or unsubstituted aryl or substituted or unsubstituted heterocycle,
- 3) halogen,
- 4) OR^6 ,
- 5) NR^6R^7 ,
- 30 6) CN ,
- 7) NO_2 ,
- 8) CF_3 ;
- 9) $-\text{S}(\text{O})_m\text{R}^4$,
- 10) $-\text{OS}(\text{O})_2\text{R}^4$,

- 11) $-\text{C}(\text{O})\text{NR}^6\text{R}^7$,
- 12) $-\text{C}(\text{O})\text{OR}^6$, or
- 13) $\text{C}_3\text{-C}_6$ cycloalkyl;

5 Z^2 is selected from a bond, unsubstituted or substituted aryl and unsubstituted or substituted heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with one or more of:

- 1) C_{1-8} alkyl, C_{2-8} alkenyl or C_{2-8} alkynyl, unsubstituted or substituted with:
 - 10 a) C_{1-4} alkoxy,
 - b) NR^6R^7 ,
 - c) C_{3-6} cycloalkyl,
 - d) aryl or heterocycle,
 - e) HO ,
 - 15 f) $-\text{S}(\text{O})_m\text{R}^4$,
 - g) $-\text{C}(\text{O})\text{NR}^6\text{R}^7$, or
 - h) C_{1-4} perfluoroalkyl;
- 2) substituted or unsubstituted aryl or substituted or unsubstituted heterocycle,
- 20 3) halogen,
- 4) OR^6 ,
- 5) NR^6R^7 ,
- 6) CN ,
- 7) NO_2 ,
- 25 8) CF_3 ,
- 9) $-\text{S}(\text{O})_m\text{R}^4$,
- 10) $-\text{OS}(\text{O})_2\text{R}^4$,
- 11) $-\text{C}(\text{O})\text{NR}^6\text{R}^7$,
- 12) $-\text{C}(\text{O})\text{OR}^6$, or
- 30 13) $\text{C}_3\text{-C}_6$ cycloalkyl;

k is 0, 1, 2, 3 or 4;

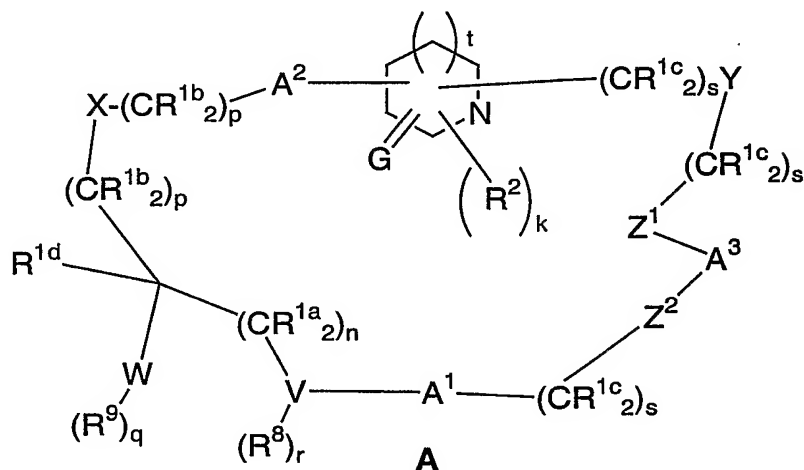
m is 0, 1 or 2;

n is 0, 1, 2, 3, 4 or 5;

- p is independently 0, 1, 2, 3, 4 or 5;
 q is 1, 2 or 3;
 r is 0, 1, 2, 3, 4 or 5;
 s is independently 0, 1, 2, 3, 4 or 5;
 5 t is 0 or 1; and
 u is independently 2, 3, 4, 5 or 6;

or a pharmaceutically acceptable salt or stereoisomer thereof.

- 10 In another embodiment of this invention, the inhibitors of prenyl-protein transferase are illustrated by the formula A:



wherein:

R^{1a}, R^{1b}, R^{1c} and R^{1d} are independently selected from:

- 15 a) hydrogen,
 b) unsubstituted or substituted aryl,
 c) unsubstituted or substituted heterocycle,
 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 e) R¹⁰O-,
 20 f) R¹¹S(O)_m-,
 g) R¹⁰C(O)NR¹⁰-,
 h) (R¹⁰)₂NC(O)-,
 i) CN,
 j) NO₂,

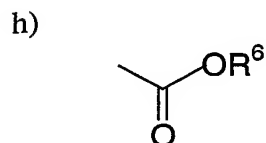
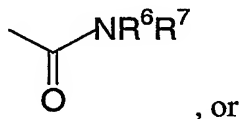
- k) $R^{10}C(O)-$,
 l) $R^{10}OC(O)-$,
 m) $-N(R^{10})_2$,
 n) $R^{11}OC(O)NR^{10}-$,
 5 o) unsubstituted or substituted C_1-C_6 alkyl,
 p) unsubstituted or substituted C_2-C_6 alkenyl, or
 q) unsubstituted or substituted C_2-C_6 alkynyl,

wherein the substituent on the substituted group is selected from unsubstituted
 or substituted C_1-C_6 alkyl, unsubstituted or substituted aryl,
 10 unsubstituted or substituted heterocycle, unsubstituted or substituted
 C_3-C_{10} cycloalkyl, unsubstituted or substituted C_2-C_6 alkenyl,
 unsubstituted or substituted C_2-C_6 alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$,
 $R^{10}C(O)NR^{10}-$, $(R^{10})_2NC(O)-$, CN , $R^{10}C(O)-$, $R^{10}OC(O)-$,
 $-N(R^{10})_2$, and $R^{11}OC(O)NR^{10}-$,

15 or two R^{1a} s, two R^{1b} s or two R^{1c} s, on the same carbon atom may be combined to
 form $-(CH_2)_u-$;

R^2 is independently selected from

- 20 a) hydrogen,
 b) unsubstituted or substituted C_{1-8} alkyl,
 c) unsubstituted or substituted C_{2-8} alkenyl,
 d) unsubstituted or substituted C_{2-8} alkynyl,
 e) unsubstituted or substituted aryl,
 25 f) unsubstituted or substituted heterocycle,
 g)



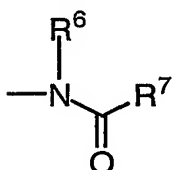
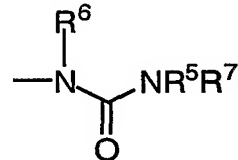
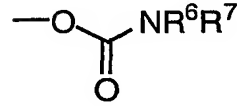
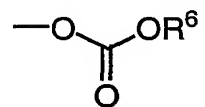
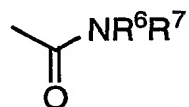
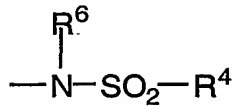
30 wherein the substituted group is substituted with one or more of:

- 1) aryl or heterocycle, unsubstituted or substituted with:

5

- a) C₁₋₄ alkyl,
 - b) (CH₂)_pOR⁶,
 - c) (CH₂)_pNR⁶R⁷,
 - d) halogen,
 - e) CN,
- 2) C₃₋₆ cycloalkyl,
 - 3) OR⁶,
 - 4) SR⁴, S(O)R⁴, SO₂R⁴,

10

- 5) —NR⁶R⁷ ,
- 6)  ,
- 7)  ,
- 8)  ,
- 9)  ,
- 10)  ,
- 11) —SO₂—NR⁶R⁷ ,
- 12)  ,

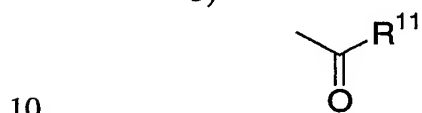


15) N₃, or

16) F; or

R⁴ is selected from C₁₋₄ alkyl, C₃₋₆ cycloalkyl, heterocycle, aryl, unsubstituted or substituted with:

- 5 1) C₁₋₄ alkoxy,
 2) aryl or heterocycle,
 3) halogen,
 4) HO,
 5)



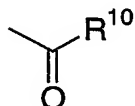
- 6) SO₂R¹¹,
 7) N(R¹⁰)₂, or
 8) C₁₋₄ perfluoroalkyl;

15

R⁵, R⁶ and R⁷ are independently selected from:

- a) hydrogen,
 b) R¹⁰C(O)-,
 c) R¹⁰OC(O)-, or
 20 d) C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₆ cycloalkyl,
 heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl,
 unsubstituted or substituted with one or more substituents selected
 from:
 1) R¹⁰O-,
 25 2) aryl or heterocycle,

- 3) halogen,
- 4) $R^{10}C(O)NR^{10}-$,
- 5)



5

- 6) SO_2R^{11} ,
- 7) $N(R^{10})_2$,
- 8) C_{3-6} cycloalkyl,
- 9) C_1-C_6 perfluoroalkyl,
- 10) $R^{10}OC(O)-$,
- 11) $R^{11}OC(O)NR^{10}-$,
- 12) CN, and
- 13) NO_2 ; or

10

15 R^6 and R^7 may be joined in a ring; and independently,

R^5 and R^7 may be joined in a ring;

R^8 is independently selected from:

20

- a) hydrogen,
- b) unsubstituted or substituted aryl,
- c) unsubstituted or substituted heterocycle,
- d) unsubstituted or substituted C_3-C_{10} cycloalkyl,
- e) unsubstituted or substituted C_2-C_6 alkenyl,
- 25 f) unsubstituted or substituted C_2-C_6 alkynyl,
- g) C_1-C_6 perfluoroalkyl,
- h) halo,
- i) $R^{10}O-$,
- j) $R^{11}S(O)_m-$,
- 30 k) $R^{10}C(O)NR^{10}-$,
- l) $(R^{10})_2NC(O)-$,
- m) CN,
- n) NO_2 ,

30

- o) $R^{10}C(O)-$,
 p) $R^{10}OC(O)-$,
 q) $-N(R^{10})_2$,
 r) $R^{11}OC(O)NR^{10}-$, and
 5 s) unsubstituted or substituted C_1-C_6 alkyl, optionally substituted by unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, $(R^{10})_2NC(O)-$, CN, $R^{10}C(O)-$, $R^{10}OC(O)-$, $-N(R^{10})_2$,
 10 or $R^{10}OC(O)NR^{10}-$;

R^9 is selected from:

- a) hydrogen,
 b) unsubstituted or substituted C_2-C_6 alkenyl,
 15 c) unsubstituted or substituted C_2-C_6 alkynyl,
 d) C_1-C_6 perfluoroalkyl,
 e) halo,
 f) $R^{10}O-$,
 g) $R^{11}S(O)_m-$,
 20 h) $R^{10}C(O)NR^{10}-$,
 i) $(R^{10})_2NC(O)-$,
 j) CN,
 k) NO_2 ,
 l) $R^{10}C(O)-$,
 25 m) $R^{10}OC(O)-$,
 n) $-N(R^{10})_2$,
 o) $R^{11}OC(O)NR^{10}-$, or
 p) unsubstituted or substituted C_1-C_6 alkyl optionally substituted by C_1-C_6 perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, $(R^{10})_2NC(O)-$, CN, $R^{10}C(O)-$, $R^{10}OC(O)-$, $-N(R^{10})_2$,
 30 or $R^{11}OC(O)NR^{10}-$;

R^{10} is independently selected from

- a) hydrogen,

- 5 b) unsubstituted or substituted C₁-C₆ alkyl,
 c) unsubstituted or substituted C₁-C₃ perfluoroalkyl,
 d) unsubstituted or substituted benzyl,
 e) unsubstituted or substituted aryl, or
 f) unsubstituted or substituted heterocycle;

R¹¹ is independently selected from

- 10 a) unsubstituted or substituted C₁-C₆ alkyl,
 b) unsubstituted or substituted aryl, or
 c) unsubstituted or substituted heterocycle;

A¹ and A³ are independently selected from

- 15 a) a bond,
 b) -C(O)-,
 c) -C(O)NR¹⁰-,
 d) -NR¹⁰C(O)-,
 e) O,
 f) -N(R¹⁰)-,
 g) -S(O)₂N(R¹⁰)-,
20 h) -N(R¹⁰)S(O)₂-, or
 i) S(O)_m;

A² is selected from

- 25 a) a bond,
 b) -C(O)-,
 c) -C(O)NR¹⁰-,
 d) -NR¹⁰C(O)-,
 e) O,
 f) -N(R¹⁰)-,
30 g) -S(O)₂N(R¹⁰)-,
 h) -N(R¹⁰)S(O)₂-,
 i) -NR¹⁰C(O)NR¹⁰-, or
 j) S(O)_m;

G is selected from H₂ or O;

V is selected from pyridyl or phenyl;

5 W is a heterocycle;

X is selected from:

- a) a bond,
- b) -C(O)-,
- 10 c) -C(O)NR¹⁰-,
- d) -NR¹⁰C(O)-,
- e) -NR¹⁰C(O)-O-,
- f) -O-C(O)NR¹⁰-,
- 15 g) -NR¹⁰C(O)NR¹⁰-,
- h) O,
- i) -N(R¹⁰)-,
- j) -S(O)₂N(R¹⁰)-,
- k) -N(R¹⁰)S(O)₂-, or
- 20 l) S(O)_m;

Y is selected from

- a) a bond,
- b) -C(=O)-,
- c) -C(O)NR¹⁰-,
- 25 d) -C(O)O-, or
- e) -S(=O)_m;

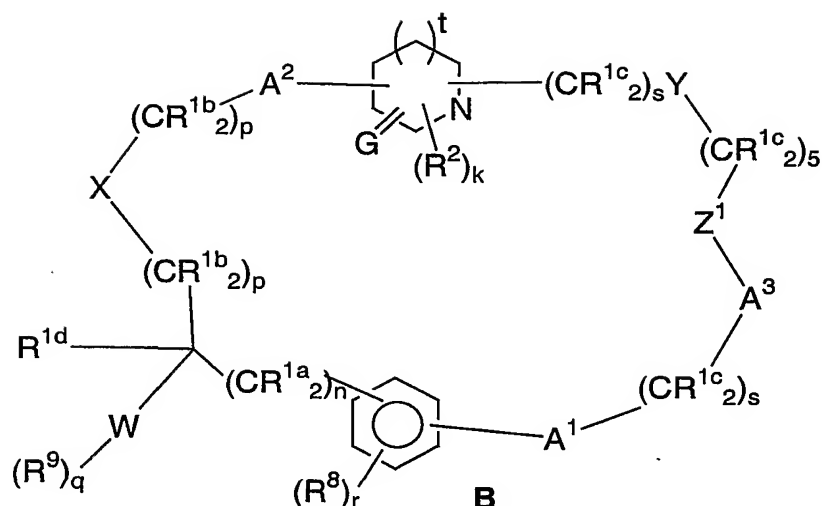
Z¹ is selected from unsubstituted or substituted aryl and unsubstituted or substituted heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with
30 one or more of:

- 1) C₁₋₈ alkyl, C₂₋₈ alkenyl or C₂₋₈ alkynyl, unsubstituted or substituted
with:
 - a) C₁₋₄ alkoxy,
 - b) NR⁶R⁷,

- 5
- c) C₃₋₆ cycloalkyl,
 - d) aryl or heterocycle,
 - e) HO,
 - f) -S(O)_mR⁴,
 - g) -C(O)NR⁶R⁷, or
 - h) C₁₋₄ perfluoroalkyl;
- 10
- 2) substituted or unsubstituted aryl or substituted or unsubstituted heterocycle,
 - 3) halogen,
 - 4) OR⁶,
 - 5) NR⁶R⁷,
 - 6) CN,
 - 7) NO₂,
 - 8) CF₃;
- 15
- 9) -S(O)_mR⁴,
 - 10) -OS(O)₂R⁴,
 - 11) -C(O)NR⁶R⁷,
 - 12) -C(O)OR⁶, or
 - 13) C₃₋₆ cycloalkyl;
- 20
- Z² is selected from a bond, unsubstituted or substituted aryl and unsubstituted or substituted heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with one or more of:
- 25
- 1) C₁₋₈ alkyl, C₂₋₈ alkenyl or C₂₋₈ alkynyl, unsubstituted or substituted with:
 - a) C₁₋₄ alkoxy,
 - b) NR⁶R⁷,
 - c) C₃₋₆ cycloalkyl,
 - d) aryl or heterocycle,
 - e) HO,
 - f) -S(O)_mR⁴,
 - g) -C(O)NR⁶R⁷, or
 - h) C₁₋₄ perfluoroalkyl;
- 30

- 2) substituted or unsubstituted aryl or substituted or unsubstituted heterocycle,
 3) halogen,
 4) OR⁶,
 5) NR⁶R⁷,
 6) CN,
 7) NO₂,
 8) CF₃,
 9) -S(O)_mR⁴,
 10) -OS(O)₂R⁴,
 11) -C(O)NR⁶R⁷,
 12) -C(O)OR⁶, or
 13) C₃-C₆ cycloalkyl;
- 15 k is 0, 1, 2, 3 or 4;
 m is 0, 1 or 2;
 n is 0, 1, 2, 3, 4 or 5;
 p is independently 0, 1, 2, 3, 4 or 5;
 q is 1, 2 or 3;
 20 r is 0, 1, 2, 3, 4 or 5;
 s is independently 0, 1, 2, 3, 4 or 5;
 t is 0 or 1; and
 u is independently 2, 3, 4, 5 or 6;
- 25 or a pharmaceutically acceptable salt or stereoisomer thereof.

In another embodiment of this invention, the inhibitors of prenyl-protein transferase are illustrated by the formula B:



wherein:

R^{1a}, R^{1b} and R^{1c} are independently selected from:

- 5 a) hydrogen,
b) unsubstituted or substituted aryl,
c) unsubstituted or substituted heterocycle,
d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
e) -N(R¹⁰)₂,
10 f) unsubstituted or substituted C₂-C₆ alkenyl
g) -OR¹⁰, or
h) unsubstituted or substituted C₁-C₆ alkyl,
wherein the substituent on the substituted group is selected from
unsubstituted or substituted aryl, heterocycle, C₃-C₁₀ cycloalkyl,
15 C₂-C₆ alkenyl, R¹⁰O-, and -N(R¹⁰)₂,

or two R^{1a}s, two R^{1b}s or two R^{1c}s, on the same carbon atom may be combined to form -(CH₂)_n;

- 20 R1d is selected from:
- a) hydrogen,
 - b) unsubstituted or substituted aryl,
 - c) unsubstituted or substituted heterocycle,

- 5
- d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 - e) R¹⁰O-,
 - f) R¹¹S(O)_m-,
 - g) R¹⁰C(O)NR¹⁰-,
 - h) (R¹⁰)₂NC(O)-,
 - i) CN,
 - j) NO₂,
 - k) R¹⁰C(O)-,
 - l) R¹⁰OC(O)-,
 - 10 m) -N(R¹⁰)₂,
 - n) R¹¹OC(O)NR¹⁰-,
 - o) unsubstituted or substituted C₁-C₆ alkyl,
 - p) unsubstituted or substituted C₂-C₆ alkenyl, or
 - q) unsubstituted or substituted C₂-C₆ alkynyl,

15

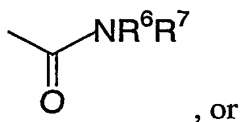
wherein the substituent on the substituted group is selected from unsubstituted or substituted aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, and R¹¹OC(O)NR¹⁰-;

20

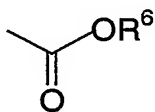
R² is independently selected from

- a) hydrogen,
- b) unsubstituted or substituted C₁-8 alkyl,
- c)

25



d)



wherein the substituted group is substituted with one or more of:

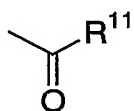
30

- 1) aryl or heterocycle, unsubstituted or substituted with:
 - a) C₁-4 alkyl,
 - b) (CH₂)_pOR⁶,

- 5
- c) $(\text{CH}_2)_p\text{NR}^6\text{R}^7$,
 - d) halogen,
 - e) CN,
 - 2) OR^6 ,
 - 3) SR^4 , $\text{S}(\text{O})\text{R}^4$, SO_2R^4 , or
 - 4) $-\text{NR}^6\text{R}^7$;

R^4 is selected from C_{1-4} alkyl, or C_{3-6} cycloalkyl, unsubstituted or substituted with:

- 10
- 1) C_{1-4} alkoxy,
 - 2) aryl or heterocycle,
 - 3) halogen,
 - 4) HO,
 - 5)

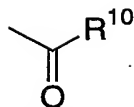


- 15
- 6) SO_2R^{11} ,
 - 7) $\text{N}(\text{R}^{10})_2$, or
 - 8) C_{1-4} perfluoroalkyl;

20 R^6 and R^7 are independently selected from:

- a) hydrogen,
 - b) $\text{R}^{10}\text{C}(\text{O})-$,
 - c) $\text{R}^{10}\text{OC}(\text{O})-$, or
 - d) $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl, C_{3-6} cycloalkyl,
- 25 heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with one or more substituents selected from:
- 1) $\text{R}^{10}\text{O}-$,
 - 2) aryl or heterocycle,
 - 3) halogen,
 - 4) $\text{R}^{10}\text{C}(\text{O})\text{NR}^{10}-$,
- 30

5)

6) SO_2R^{11} ,7) $\text{N}(\text{R}^{10})_2$,

5

8) $\text{C}_3\text{-C}_6$ cycloalkyl,9) $\text{C}_1\text{-C}_6$ perfluoroalkyl,10) $\text{R}^{10}\text{OC}(\text{O})-$,11) $\text{R}^{11}\text{OC}(\text{O})\text{NR}^{10}-$,12) CN , and

10

13) NO_2 ; or

R^6 and R^7 may be joined in a ring;

R^8 is independently selected from:

15

a) hydrogen,

b) unsubstituted or substituted aryl,

c) unsubstituted or substituted heterocycle,

d) unsubstituted or substituted $\text{C}_3\text{-C}_{10}$ cycloalkyl,e) unsubstituted or substituted $\text{C}_2\text{-C}_6$ alkenyl,

20

f) unsubstituted or substituted $\text{C}_2\text{-C}_6$ alkynyl,g) $\text{C}_1\text{-C}_6$ perfluoroalkyl,

h) halo,

i) $\text{R}^{10}\text{O}-$,j) $\text{R}^{11}\text{S}(\text{O})_m-$,

25

k) $\text{R}^{10}\text{C}(\text{O})\text{NR}^{10}-$,l) $(\text{R}^{10})_2\text{NC}(\text{O})-$,m) CN ,n) NO_2 ,o) $\text{R}^{10}\text{C}(\text{O})-$,

30

p) $\text{R}^{10}\text{OC}(\text{O})-$,q) $-\text{N}(\text{R}^{10})_2$,r) $\text{R}^{11}\text{OC}(\text{O})\text{NR}^{10}-$, ors) unsubstituted or substituted $\text{C}_1\text{-C}_6$ alkyl, optionally substituted

by unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹⁰OC(O)NR¹⁰-;

R⁹ is selected from:

- a) hydrogen,
- b) C₁-C₆ perfluoroalkyl,
- 10 c) R¹⁰O-,
- d) R¹⁰C(O)NR¹⁰-,
- e) (R¹⁰)₂NC(O)-, or
- f) unsubstituted or substituted C₁-C₆ alkyl optionally substituted by C₁-C₆ perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-;
- 15

R¹⁰ is independently selected from

- a) hydrogen,
- 20 b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₁-C₃ perfluoroalkyl,
- d) unsubstituted or substituted benzyl,
- e) unsubstituted or substituted aryl, or
- f) unsubstituted or substituted heterocycle;

25

R¹¹ is independently selected from

- a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted aryl, or
- c) unsubstituted or substituted heterocycle;

30

A¹ and A³ are independently selected from

- a) a bond,
- b) -C(O)-,
- c) -C(O)NR¹⁰-,
- 35 d) -NR¹⁰C(O)-,

- 5 e) O,
f) -N(R¹⁰)-,
g) -S(O)₂N(R¹⁰)-,
h) -N(R¹⁰)S(O)₂-, or
i) S(O)_m;

A² is selected from

- 10 a) a bond,
b) -C(O)-,
c) -C(O)NR¹⁰-,
d) -NR¹⁰C(O)-,
e) O,
f) -N(R¹⁰)-,
g) -S(O)₂N(R¹⁰)-,
15 h) -N(R¹⁰)S(O)₂-,
i) -NR¹⁰C(O)NR¹⁰-, or
j) S(O)_m;

G is selected from H₂ or O;

20

W is a heterocycle selected from imidazolyl, pyridyl, or triazolyl;

X is selected from:

- 25 a) a bond,
b) -C(O)-,
c) -C(O)NR¹⁰-,
d) -NR¹⁰C(O)-,
e) -NR¹⁰C(O)-O-,
f) -O-C(O)NR¹⁰-,
30 g) -NR¹⁰C(O)NR¹⁰-,
h) O,
i) -N(R¹⁰)-,
j) -S(O)₂N(R¹⁰)-,
k) -N(R¹⁰)S(O)₂-, or

l) $S(O)_m$;

Y is selected from

- 5 a) a bond,
 b) $-C(=O)-$,
 c) $-C(O)NR^{10}-$,
 d) $-C(O)O-$, or
 e) $-S(=O)_m$;

10 Z^1 is selected from unsubstituted or substituted aryl and unsubstituted or substituted heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with one or more of:

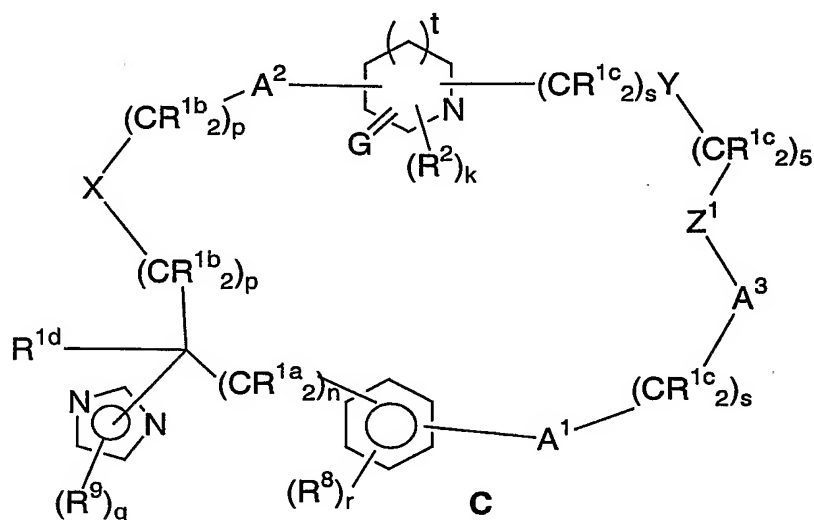
- 1) C_1 -8 alkyl, C_2 -8 alkenyl or C_2 -8 alkynyl, unsubstituted or substituted with:
- 15 a) C_1 -4 alkoxy,
 b) NR^6R^7 ,
 c) C_3 -6 cycloalkyl,
 d) aryl or heterocycle,
 e) HO,
 f) $-S(O)_mR^4$,
 g) $-C(O)NR^6R^7$, or
 h) C_1 -4 perfluoroalkyl;
- 2) substituted or unsubstituted aryl or substituted or unsubstituted heterocycle,
- 25 3) halogen,
 4) OR^6 ,
 5) NR^6R^7 ,
 6) CN,
 7) NO_2 ,
- 30 8) CF_3 ;
 9) $-S(O)_mR^4$,
 10) $-OS(O)_2R^4$,
 11) $-C(O)NR^6R^7$,
 12) $-C(O)OR^6$, or

13) C₃-C₆ cycloalkyl;

- k is 0, 1, 2, 3 or 4;
 m is 0, 1 or 2;
 5 n is 0, 1, 2, 3, 4 or 5;
 p is independently 0, 1, 2, 3, 4 or 5;
 q is 1, 2 or 3;
 r is 0, 1, 2, 3, 4 or 5;
 s is independently 0, 1, 2, 3, 4 or 5;
 10 t is 0 or 1; and
 u is independently 2, 3, 4, 5 or 6;

or a pharmaceutically acceptable salt or stereoisomer thereof.

- 15 In a further preferred embodiment of this invention, the inhibitors of prenyl-protein transferase are illustrated by the formula C:



wherein:

- 20 R^{1a}, R^{1b} and R^{1c} are independently selected from:
- hydrogen,
 - unsubstituted or substituted aryl,
 - unsubstituted or substituted heterocycle,

- d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 e) -N(R¹⁰)₂,
 f) unsubstituted or substituted C₂-C₆ alkenyl
 g) -OR¹⁰, or
 5 h) unsubstituted or substituted C₁-C₆ alkyl,
 wherein the substituent on the substituted group is selected from
 unsubstituted or substituted aryl, heterocycle, C₃-C₁₀ cycloalkyl,
 C₂-C₆ alkenyl, R¹⁰O-, and -N(R¹⁰)₂,

10 or two R^{1a}s, two R^{1b}s or two R^{1c}s, on the same carbon atom may be combined to
 form -(CH₂)_u-;

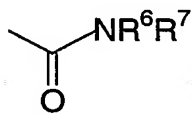
R^{1d} is selected from:

- a) hydrogen,
 15 b) unsubstituted or substituted aryl,
 c) unsubstituted or substituted heterocycle,
 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 e) R¹⁰O-,
 f) -N(R¹⁰)₂, or
 20 g) unsubstituted or substituted C₁-C₆ alkyl,
 wherein the substituent on the substituted group is selected from unsubstituted
 or substituted aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl,
 R¹⁰O-, R¹⁰C(O)NR¹⁰-, (R¹⁰)₂NC(O)-, R¹⁰C(O)-, R¹⁰OC(O)-,
 -N(R¹⁰)₂, and R¹¹OC(O)-NR¹⁰-;

25

R² is independently selected from

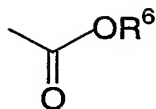
- a) hydrogen,
 b) unsubstituted or substituted C₁₋₈ alkyl,
 c)



30

, or

d)



wherein the substituted group is substituted with one or more of:

- 1) aryl or heterocycle, unsubstituted or substituted with:
 - a) C₁₋₄ alkyl,
 - b) (CH₂)_pOR⁶,
 - c) (CH₂)_pNR⁶R⁷,
 - d) halogen,
 - e) CN,
- 2) OR⁶,
- 3) SR⁴, S(O)R⁴, SO₂R⁴, or
- 4) -NR⁶R⁷;

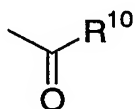
R⁴ is selected from C₁₋₄ alkyl, or C₃₋₆ cycloalkyl, unsubstituted or substituted with:

- 1) C₁₋₄ alkoxy,
- 2) aryl or heterocycle,
- 3) halogen, or
- 4) -OH;

R⁶ and R⁷ are independently selected from:

- a) hydrogen, or
- b) C_{1-C6} alkyl, C_{2-C6} alkenyl, C_{2-C6} alkynyl, C₁₋₆ perfluoroalkyl, C₃₋₆ cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with one or more substituents selected from:

- 1) R¹⁰O-,
- 2) aryl or heterocycle,
- 3) halogen,
- 4) R¹⁰C(O)NR¹⁰-,
- 5)



- 6) SO_2R^{11} ,
- 7) $\text{N}(\text{R}^{10})_2$, or
- 8) $\text{C}_3\text{-C}_6$ cycloalkyl, or

5 R^6 and R^7 may be joined in a ring;

R^8 is independently selected from:

- a) hydrogen,
- b) unsubstituted or substituted aryl,
- 10 c) unsubstituted or substituted heterocycle,
- d) unsubstituted or substituted $\text{C}_3\text{-C}_{10}$ cycloalkyl,
- e) unsubstituted or substituted $\text{C}_2\text{-C}_6$ alkenyl,
- f) unsubstituted or substituted $\text{C}_2\text{-C}_6$ alkynyl,
- g) $\text{C}_1\text{-C}_6$ perfluoroalkyl,
- 15 h) halo,
- i) $\text{R}^{10}\text{O}-$,
- j) $\text{R}^{11}\text{S}(\text{O})_m-$,
- k) $\text{R}^{10}\text{C}(\text{O})\text{NR}^{10}-$,
- l) $(\text{R}^{10})_2\text{NC}(\text{O})-$,
- 20 m) CN ,
- n) NO_2 ,
- o) $\text{R}^{10}\text{C}(\text{O})-$,
- p) $\text{R}^{10}\text{OC}(\text{O})-$,
- q) $-\text{N}(\text{R}^{10})_2$,
- 25 r) $\text{R}^{11}\text{OC}(\text{O})\text{NR}^{10}-$, and
- s) unsubstituted or substituted $\text{C}_1\text{-C}_6$ alkyl, optionally substituted by unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, $\text{C}_3\text{-C}_{10}$ cycloalkyl, $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl, $\text{C}_1\text{-C}_6$ perfluoroalkyl, F, Cl, Br, $\text{R}^{10}\text{O}-$, $\text{R}^{11}\text{S}(\text{O})_m-$, $\text{R}^{10}\text{C}(\text{O})\text{NR}^{10}-$, $(\text{R}^{10})_2\text{NC}(\text{O})-$, CN , $\text{R}^{10}\text{C}(\text{O})-$, $\text{R}^{10}\text{OC}(\text{O})-$, $-\text{N}(\text{R}^{10})_2$, or $\text{R}^{10}\text{OC}(\text{O})\text{NR}^{10}-$;
- 30

R^9 is selected from:

- a) hydrogen,

- b) C₁-C₆ perfluoroalkyl, or
 c) unsubstituted or substituted C₁-C₆ alkyl optionally substituted by
 C₁-C₆ perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-,
 (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or
 5 R¹¹OC(O)NR¹⁰-;

R¹⁰ is independently selected from

- a) hydrogen,
 b) unsubstituted or substituted C₁-C₆ alkyl,
 10 c) unsubstituted or substituted C₁-C₃ perfluoroalkyl,
 d) unsubstituted or substituted benzyl,
 e) unsubstituted or substituted aryl, or
 f) unsubstituted or substituted heterocycle;

15 R¹¹ is independently selected from

- a) unsubstituted or substituted C₁-C₆ alkyl,
 b) unsubstituted or substituted aryl, or
 c) unsubstituted or substituted heterocycle;

20 A¹ and A³ are independently selected from

- a) a bond,
 b) -C(O)-,
 c) -C(O)NR¹⁰-,
 d) -NR¹⁰C(O)-,
 25 e) O,
 f) -N(R¹⁰)-,
 g) -S(O)₂N(R¹⁰)-,
 h) -N(R¹⁰)S(O)₂-, or
 i) S(O)_m;

30

A² is selected from

- a) a bond,
 b) -C(O)-,
 c) -C(O)NR¹⁰-,

- 5
- d) $\text{-NR}^{10}\text{C(O)-}$,
 - e) O ,
 - f) $\text{-N(R}^{10})\text{-}$,
 - g) $\text{-S(O)}_2\text{N(R}^{10})\text{-}$,
 - h) $\text{-N(R}^{10})\text{S(O)}_2\text{-}$,
 - i) $\text{-NR}^{10}\text{C(O)NR}^{10}\text{-}$, or
 - j) S(O)_m ;

G is selected from H_2 or O;

10

X is selected from:

- 15
- a) a bond,
 - b) -C(O)- ,
 - c) $\text{-C(O)NR}^{10}\text{-}$,
 - d) $\text{-NR}^{10}\text{C(O)-}$,
 - e) $\text{-NR}^{10}\text{C(O)-O-}$,
 - f) $\text{-O-C(O)NR}^{10}\text{-}$,
 - g) $\text{-NR}^{10}\text{C(O)NR}^{10}\text{-}$,
 - h) O ,
 - 20 i) $\text{-N(R}^{10})\text{-}$,
 - j) $\text{-S(O)}_2\text{N(R}^{10})\text{-}$,
 - k) $\text{-N(R}^{10})\text{S(O)}_2\text{-}$, or
 - l) S(O)_m ;

25 Y is selected from

- 30
- a) a bond,
 - b) -C(=O)- ,
 - c) $\text{-C(O)NR}^{10}\text{-}$,
 - d) -C(O)O- , or
 - e) -S(=O)_m ;

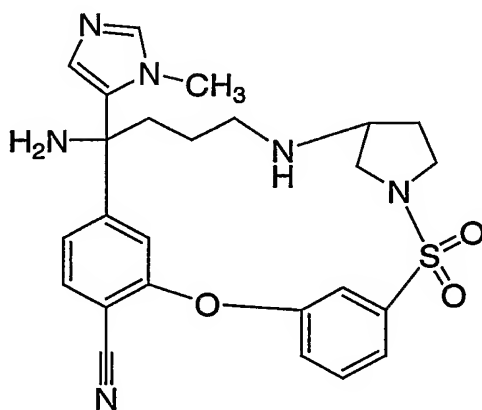
Z^1 is selected from unsubstituted or substituted aryl and unsubstituted or substituted heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with one or more of:

- 1) C₁₋₈ alkyl, C₂₋₈ alkenyl or C₂₋₈ alkynyl, unsubstituted or substituted with:
- 5 a) C₁₋₄ alkoxy,
 b) NR⁶R⁷,
 c) C₃₋₆ cycloalkyl,
 d) aryl or heterocycle,
 e) HO,
 f) -S(O)_mR⁴,
 g) -C(O)NR⁶R⁷, or
 10 h) C₁₋₄ perfluoroalkyl;
- 2) substituted or unsubstituted aryl or substituted or unsubstituted heterocycle,
 3) halogen,
 4) OR⁶,
 15 5) NR⁶R⁷,
 6) CN,
 7) NO₂,
 8) CF₃;
 9) -S(O)_mR⁴,
 20 10) -OS(O)₂R⁴,
 11) -C(O)NR⁶R⁷,
 12) -C(O)OR⁶, or
 13) C_{3-C6} cycloalkyl;
- 25 k is 0, 1, 2, 3 or 4;
 m is 0, 1 or 2;
 n is 0, 1, 2, 3, 4 or 5;
 p is independently 0, 1, 2, 3, 4 or 5;
 q is 1, 2 or 3;
 30 r is 0, 1, 2, 3, 4 or 5;
 s is independently 0, 1, 2, 3, 4 or 5;
 t is 0 or 1; and
 u is independently 2, 3, 4, 5 or 6;

or a pharmaceutically acceptable salt or stereoisomer thereof.

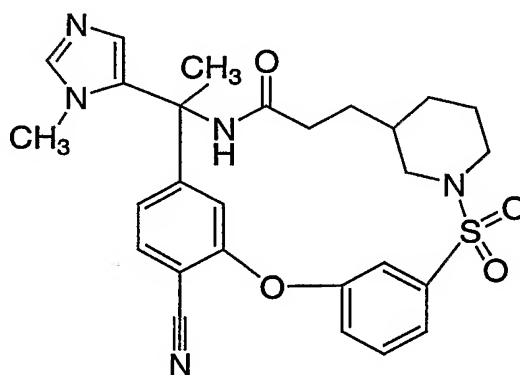
A specific example of the compounds of the instant invention is illustrated by:

5



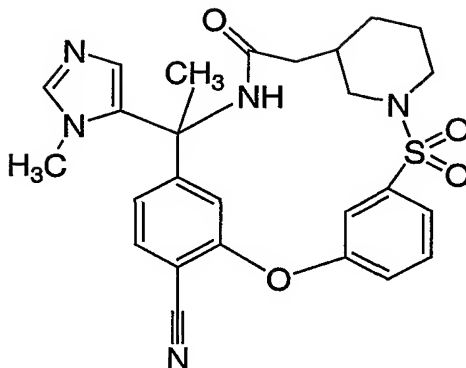
6(*R or S*)-11(*R or S*)-amino-11-(1-methyl-1H-imidazol-5-yl)-17-oxa-2-thia-3,7-diazatetracyclo [16.3.1.1^{3,6}.1^{12,16}]tetracos-1(22),12(23),13,15,18,20-hexaene-15-carbonitrile 2,2-dioxide,

10

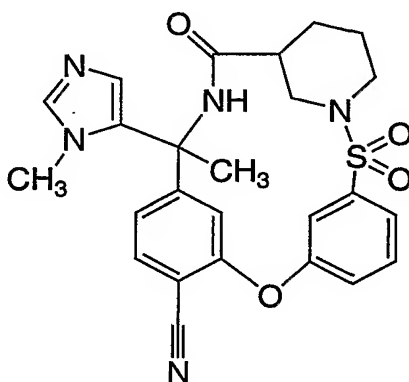


(14*R or S*,19*S or R*)-14-methyl-14-(1-methyl-1H-imidazol-5-yl)-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[17.3.1.1^{3,7}.1^{9,13}]pentacos-3(25),4,6,9(24),10,12-hexaene-10-carbonitrile 2,2-dioxide

15



(14*R* or *S*, 18*R* or *S*) -14-methyl-14-(1-methyl-1*H*-imidazol-5-yl)-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[16.3.1.1^{3,7}.1^{9,13}]tetracos-3(24),4,6,9(23),10,12-hexaene-10-carbonitrile 2,2-dioxide



(14*R* or *S*, 17*R* or *S*) -14-methyl-14-(1-methyl-1*H*-imidazol-5-yl)-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[15.3.1.1^{3,7}.1^{9,13}]tricos-3(23),4,6,9(22),10,12-hexaene-10-carbonitrile 2,2-dioxide

or a pharmaceutically acceptable salt or stereoisomer thereof.

The compounds of the present invention may have asymmetric centers, chiral axes and chiral planes, and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers, including optical isomers, being included in the present invention. (See E.L. Eliel and S.H. Wilen *Stereochemistry of Carbon Compounds* (John Wiley and Sons, New York 1994), in particular pages 1119-1190) When any variable (e.g. aryl, heterocycle, R^{1a}, R⁶ etc.) occurs more than one time in any constituent, its definition on each occurrence

is independent at every other occurrence. Also, combinations of substituents/or variables are permissible only if such combinations result in stable compounds.

As used herein, "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having from 1 to 20 carbon atoms, unless otherwise specified; "alkoxy" represents an alkyl having from 1 to 20 carbon atoms, unless otherwise indicated, attached through an oxygen bridge. "Halogen" or "halo" as used herein means fluoro, chloro, bromo and iodo.

As used herein, "cycloalkyl" is intended to include non-aromatic hydrocarbon groups having from 3 to 10 carbon atoms, unless otherwise specified. Examples of such cycloalkyl groups includes, but are not limited to, cyclopropyl, cyclobutyl, cyclohexyl, cycloheptyl, cyclooctyl, adamantyl and the like.

If no number of carbon atoms is specified, the term "alkenyl" refers to a non-aromatic hydrocarbon, straight, branched or cyclic, containing from 2 to 10 carbon atoms, unless otherwise indicated, and at least one carbon to carbon double bond. Preferably one carbon to carbon double bond is present, and up to four non-aromatic carbon-carbon double bonds may be present. Thus, "C₂-C₈ alkenyl" means an alkenyl radical having from 2 to 8 carbon atoms. Examples of such alkenyl groups include, but are not limited to, ethenyl, propenyl, butenyl and cyclohexenyl. As described above with respect to alkyl, the straight, branched or cyclic portion of the alkenyl group may contain double bonds and may be substituted if a substituted alkenyl group is indicated.

The term "alkynyl" refers to a hydrocarbon radical straight, branched or cyclic, containing from 2 to 10 carbon atoms, unless otherwise indicated, and at least one carbon to carbon triple bond. Up to three carbon-carbon triple bonds may be present. Thus, "C₂-C₈ alkynyl" means an alkynyl radical having from 2 to 8 carbon atoms. Examples of such alkynyl groups include, but are not limited to, ethynyl, propynyl and butynyl. As described above with respect to alkyl, the straight, branched or cyclic portion of the alkynyl group may contain triple bonds and may be substituted if a substituted alkynyl group is indicated.

As used herein, "aryl" is intended to mean any stable monocyclic or bicyclic carbon ring of up to 7 members in each ring, wherein at least one ring is aromatic. Examples of such aryl elements include, but are not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, biphenyl, phenanthryl, anthryl, acenaphthyl and the like.

As used herein, "aralkyl" is intended to mean an aryl moiety, as

defined above, attached through a C₁-C₆ alkyl linker, where alkyl is defined above. Examples of aralkyls include, but are not limited to, benzyl, naphthylmethyl, phenylbutyl and the like.

The term heterocycle or heterocyclic, as used herein, represents
5 a stable 5- to 7-membered monocyclic or stable 8- to 11-membered bicyclic heterocyclic ring which is either saturated or unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from the group consisting of N, O, and S, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached
10 at any heteroatom or carbon atom which results in the creation of a stable structure. The term heterocycle or heterocyclic includes heteroaryl moieties. Examples of such heterocyclic elements include, but are not limited to, azepinyl, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzo-thiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl,
15 dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, 1,3-dioxolanyl, furyl, imidazolidinyl, imidazolinyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isothiazolidinyl, morpholinyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, 2-oxopiperazinyl, 2-oxopiperdinyl, 2-oxopyrrolidinyl, piperidyl, piperazinyl, pyridyl,
20 2-pyridinonyl pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyrimidinyl, pyrrolidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiazolyl, thiazolinyl, thienofuryl, thienothienyl, thienyl and triazolyl.

As used herein, "heteroaryl" is intended to mean any stable mono-
25 cyclic or bicyclic carbon ring of up to 7 members in each ring, wherein at least one ring is aromatic and wherein from one to four carbon atoms are replaced by heteroatoms selected from the group consisting of N, O, and S. Examples of such heterocyclic elements include, but are not limited to, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl,
30 benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, furyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolyl, naphthyridinyl, oxadiazolyl, pyridyl, pyrazinyl, pyrazolyl, pyridazinyl,

pyrimidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiazolyl, thienofuryl, thienothienyl, thienyl and triazolyl.

As used herein, "heterocyclylalkyl" is intended to mean a heterocyclic moiety, as defined above, attached through a C₁-C₆ alkyl linker, where alkyl is defined above. Examples of heterocyclylalkyls include, but are not limited to, 2-pyridylmethyl, 2-imidazolylethyl, 2-quinolinylmethyl, 2-imidazolylmethyl, and the like.

As used herein, "heterocycloalkyl" is intended to mean a heterocyclic moiety, as defined above, attached through a C₁-C₆ alkyl linker, where alkyl is defined above. Examples of heterocycloalkyls include, but are not limited to, 2-morpholinylethyl, 1-piperazinylmethyl, 3-pyrrolidinylmethyl and the like.

As used herein, the terms "substituted alkyl", "substituted alkenyl", "substituted alkynyl" and "substituted alkoxy", unless otherwise defined, are intended to include the branch or straight-chain alkyl group of the specified number of carbon atoms, wherein the carbon atoms may be substituted with F, Cl, Br, I, CF₃, OCF₃, CN, N₃, NO₂, NH₂, N(C₁-C₆ alkyl)₂, oxo, OH, -O(C₁-C₆ alkyl), S(O)₀₋₂, (C₁-C₆ alkyl)S(O)₀₋₂, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₁-C₆ alkyl)S(O)₀₋₂(C₁-C₆ alkyl), C₃-C₂₀ cycloalkyl, -C(O)NH₂, HC(O)NH-(C₁-C₆ alkyl)C(O)NH-, H₂NC(O)NH-(C₁-C₆ alkyl)C(O)-, -O(C₁-C₆ alkyl)CF₃, (C₁-C₆ alkyl)OC(O)-, (C₁-C₆ alkyl)O(C₁-C₆ alkyl)-, (C₁-C₆ alkyl)C(O)₂(C₁-C₆ alkyl)-, (C₁-C₆ alkyl)OC(O)NH-, aryl, heterocycle, aralkyl, heterocyclylalkyl, heterocycloalkyl, halo-aryl, halo-aralkyl, halo-heterocycle, halo-heterocyclylalkyl, halo-heterocycloalkyl, cyano-aryl, cyano-aralkyl, cyano-heterocycle, cyano-heterocyclylalkyl, and cyano-heterocycloalkyl.

As used herein, the terms "substituted aryl", "substituted heterocycle", "substituted heteroaryl", "substituted cycloalkyl", "substituted benzyl", "substituted aralkyl" and "substituted heterocyclylalkyl", "substituted heterocycloalkyl" unless otherwise defined, are intended to include the cyclic group containing from 1 to 3 substituents in addition to the point of attachment to the rest of the compound. Such substituents are preferably selected from the group which includes but is not limited to F, Cl, Br, I, CF₃, OCF₃, NH₂, N(C₁-C₆ alkyl)₂, NO₂, CN, N₃, C₁-C₂₀ alkyl, C₃-C₂₀ cycloalkyl, -OH, -O(C₁-C₆ alkyl), S(O)₀₋₂, (C₁-C₆ alkyl)S(O)₀₋₂, (C₁-C₆ alkyl)S(O)₀₋₂(C₁-C₆ alkyl)-, -C(O)NH₂, HC(O)NH-, (C₁-C₆ alkyl)C(O)NH-, H₂NC(O)NH-, (C₁-C₆ alkyl)C(O)-, (C₁-C₆ alkyl)OC(O)-, (C₁-C₆ alkyl)O(C₁-C₆ alkyl)-, (C₁-C₆)C(O)₂(C₁-C₆ alkyl)-, (C₁-C₆ alkyl)OC(O)NH-, aryl, heterocycle, aralkyl, heterocyclylalkyl, heterocycloalkyl, halo-aryl, halo-aralkyl, halo-heterocycle,

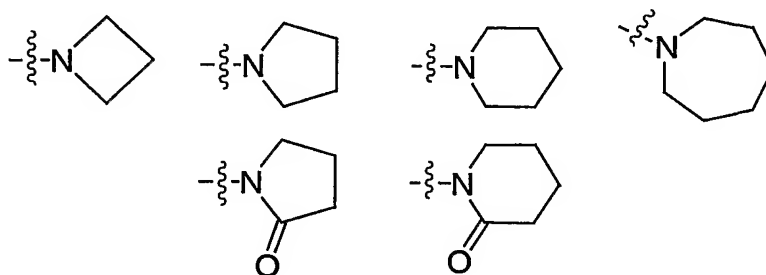
halo-heterocyclalkyl, halo-heterocycloalkyl, cyano-aryl, cyano-alkyl, cyano-heterocycle, cyano-heterocyclalkyl, and cyano-heterocycloalkyl.

Preferably, as used herein in the definition of R⁶ and R⁷, the substituted C₁₋₆ alkyl, substituted C₂₋₆ alkenyl, substituted C₂₋₆ alkynyl, substituted C₃₋₆ cycloalkyl, substituted aroyl, substituted aryl, substituted heteroaroyl, substituted arylsulfonyl, substituted heteroarylsulfonyl and substituted heterocycle, include moieties containing from 1 to 3 substituents in addition to the point of attachment to the rest of the compound.

The moiety formed when, in the definition of R^{1a}, R^{1b}, and R^{1c}, two R^{1a}s, two R^{1b}s, or two R^{1c}s, on the same carbon atom are combined to form -(CH₂)_u- is illustrated by the following:



The moiety formed when (in the definition of R⁵, R⁶ and R⁷). R⁶ and R⁷ or R⁵ and R⁷ are joined to form a ring, is illustrated by, but not limited to, the following:



Lines drawn into the ring systems from substituents (such as from R², R⁸, R⁹ etc.) indicate that the indicated bond may be attached to any of the substitutable ring carbon or nitrogen atoms.

Preferably, R^{1a} and R^{1b} are independently selected from: hydrogen, -N(R¹⁰)₂, R¹⁰C(O)NR¹⁰- or unsubstituted or substituted C₁-C₆ alkyl.

Preferably, R^{1c} is independently selected from: hydrogen, or unsubstituted or substituted C₁-C₆ alkyl.

Preferably, R^{1d} is selected from -N(R¹⁰)₂, OR¹⁰, unsubstituted or substituted C₁-C₆ alkyl, unsubstituted or substituted aryl, or unsubstituted or substituted heterocycle.

5 Preferably, R² is selected from: hydrogen and unsubstituted or substituted C₁-C₆ alkyl.

Preferably, R⁶ and R⁷ are selected from: hydrogen, unsubstituted or substituted C₁-C₆ alkyl, unsubstituted or substituted aryl and unsubstituted or substituted cycloalkyl.

10 Preferably, R⁸ is selected from hydrogen, halo, unsubstituted or substituted C₁-C₆ alkyl, unsubstituted or substituted C₁-C₆ alkoxy, unsubstituted or substituted aryl, CN, NO₂, R¹⁰C(O)NR¹⁰-, -OR¹⁰ and (R¹⁰)₂NC(O)-. Most preferably, r is 1 to 3 and at least one R⁸ is CN.

Preferably, R⁹ is hydrogen or unsubstituted or substituted C₁-C₆ alkyl. Most preferably, R⁹ is methyl.

15 Preferably, A¹ and A² are independently selected from: a bond, C(=O), -C(O)NR¹⁰-, -NR¹⁰C(O)-, O, -N(R¹⁰)-, -S(O)_m, -S(O)₂N(R¹⁰)-, and -N(R¹⁰)S(O)₂-. Most preferably, A¹ is O and A² is selected from a bond, -S(O)_m, or C(=O)-.

20 Preferably, V is selected from heteroaryl and aryl. More preferably, V is phenyl or pyridyl.

Preferably, X and Y are independently selected from: a bond, -S(=O)_m, -C(O)NR¹⁰-, -NR¹⁰C(O)-, -S(O)₂N(R¹⁰)-, -N(R¹⁰)S(O)₂-, and -C(=O)-. Most preferably, X and Y are bonds.

25 Preferably, Z¹ and Z² are independently selected from unsubstituted or substituted phenyl, unsubstituted or substituted naphthyl, unsubstituted or substituted pyridyl, unsubstituted or substituted furanyl and unsubstituted or substituted thienyl. More preferably, Z¹ is selected from unsubstituted or substituted phenyl and unsubstituted or substituted naphthyl. More preferably, Z² is selected from a bond and unsubstituted or substituted phenyl.

30 Preferably, W is selected from imidazolyl, oxazolyl, pyridyl, and triazolyl. More preferably, W is selected from imidazolyl and pyridyl. Most preferably, W is imidazolyl.

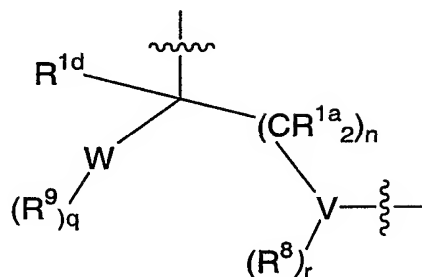
Preferably, n is 0, 1, or 2.

Preferably, r is 1 or 2.

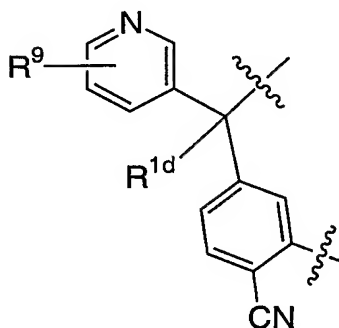
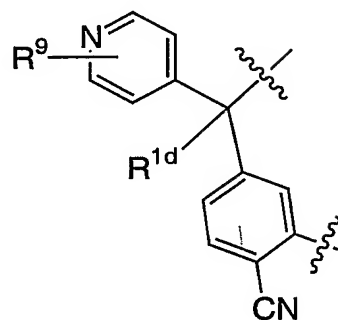
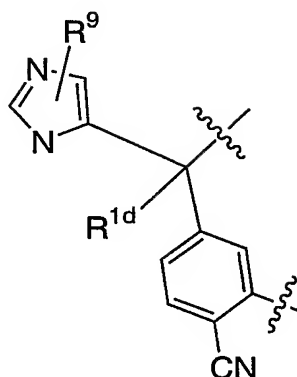
Preferably p is 0, 1, 2 or 3.

Preferably s is 0 or 1.

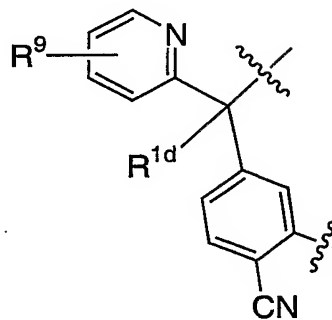
Preferably, the moiety



5 is selected from:



and



10 It is intended that the definition of any substituent or variable (e.g., R¹ᵃ, R⁹, n, etc.) at a particular location in a molecule be independent of its definitions elsewhere in that molecule. Thus, -N(R¹⁰)₂ represents -NHH, -NHCH₃, -NHC₂H₅, etc. It is understood that substituents and substitution patterns on the compounds of the instant invention can be selected by one of ordinary skill in the art

to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art, as well as those methods set forth below, from readily available starting materials.

The pharmaceutically acceptable salts of the compounds of this invention include the conventional non-toxic salts of the compounds of this invention as formed, e.g., from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like: and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like.

The pharmaceutically acceptable salts of the compounds of this invention can be synthesized from the compounds of this invention which contain a basic moiety by conventional chemical methods. Generally, the salts are prepared either by ion exchange chromatography or by reacting the free base with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents.

Abbreviations which may be used in the description of the chemistry and in the Examples that follow include:

Ac ₂ O	Acetic anhydride;
AIBN	2,2'-Azobisisobutyronitrile;
BINAP	2,2'-Bis(diphenylphosphino)-1,1' binaphthyl;
Bn	Benzyl;
BOC/Boc	t-Butoxycarbonyl;
CBz	Carbobenzyloxy;
DBAD	Di- <i>tert</i> -butyl azodicarboxylate;
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene;
DCE	1,2-Dichloroethane;
DIEA	<i>N,N</i> -Diisopropylethylamine;
DMAP	4-Dimethylaminopyridine;
DME	1,2-Dimethoxyethane;
DMF	<i>N,N</i> -Dimethylformamide;
DMSO	Methyl sulfoxide;

	DPPA	Diphenylphosphoryl azide;
	DTT	Dithiothreitol;
	EDC	1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide-hydrochloride;
	EDTA	Ethylenediaminetetraacetic acid;
5	Et ₂	Diethyl ether;
	Et ₃ N	Triethylamine;
	EtOAc	Ethyl acetate;
	EtOH	Ethanol;
	FAB	Fast atom bombardment;
10	HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid;
	HOBT	1-Hydroxybenzotriazole hydrate;
	HOBT	3-Hydroxy-1,2,2-benzotriazin-4(3 <i>H</i>)-one;
	HPLC	High-performance liquid chromatography;
	LAH	Lithium aluminum hydride;
15	MCPBA	<i>m</i> -Chloroperoxybenzoic acid;
	Me	Methyl;
	MeOH	Methanol;
	Ms	Methanesulfonyl;
	MsCl	Methanesulfonyl chloride;
20	<i>n</i> -Bu	<i>n</i> -butyl;
	<i>n</i> -Bu ₃ P	Tri- <i>n</i> -butylphosphine;
	NaHMDS	Sodium bis(trimethylsilyl)amide;
	NBS	<i>N</i> -Bromosuccinimide;
	Pd ₂ (dba) ₂	Tris(dibenzylideneacetone)dipalladium (0)
25	Ph	phenyl;
	PMSF	<i>p</i> -Toluenesulfonyl chloride;
	Py or pyr	Pyridine;
	PYBOP	Benzotriazole-1-yl-oxy-trispyrrolidinophosphonium hexafluorophosphate;
30	RT	room temperature;
	<i>t</i> -Bu	<i>tert</i> -Butyl;
	TBAF	Tetrabutylammoniumfluoride;
	RPLC	Reverse Phase Liquid Chromatography;
	TBSCl	<i>tert</i> -Butyldimethylsilyl chloride;
35	TFA	Trifluoroacetic acid;

THF Tetrahydrofuran;
TIPS Triisopropylsilyl;
TMS Tetramethylsilane; and
Tr Trityl.

5

Reactions used to generate the compounds of this invention are prepared by employing reactions as shown in the Schemes 1-11, in addition to other standard manipulations such as ester hydrolysis, cleavage of protecting groups, etc., as may be known in the literature or exemplified in the experimental procedures.

10

These reactions may be employed in a linear sequence to provide the compounds of the invention or they may be used to synthesize fragments which are subsequently joined by the reactions described in the Schemes. The procedures discussed and illustrated in the following schemes and synopsis may be used in the preparation of the compounds of the instant invention, for either (*R*) or (*S*)

15

stereochemistry.

Synopsis of Schemes

Unless otherwise indicated, variable *n* is defined as 0 to 6, variable *p* is defined as 0 to 4, and variable *t* is defined as 0 or 1. For simplicity, the number of substitutions possible on a ring has not been included. For example, R^8 in the schemes represents $(R^8)_t$ in formula I. In the following schemes, substituent *Z* represents Z^1 or Z^2 of formula I and substituent *R* represents the optional substitutions provided in the definition of Z^1 or Z^2 of formula I. Additionally,



represents substituent *W* designated in formula I.

The requisite intermediates are in some cases commercially available, or can be prepared according to literature procedures. Schemes 1-4 outline the syntheses of various aminopyrrolidine or aminopiperidine intermediates used in the syntheses of the macrocyclic compounds of the instant invention. Boc-protected amino heterocycles **1** and **6**, available commercially or by procedures known to those

30

skilled in the art, can be sulfonylated as in Schemes 1 and 2 to give compounds **2** and **7**. Compounds **2** and **7** can then deprotected to give intermediates **5** and **8**, respectively, which are used in Scheme 5, for example, to form the macrocyclic compounds of the instant invention.

5 In Scheme 3, the boc-protected amino heterocycle **6** is reductively alkylated with a suitably substituted aldehyde **B** to give intermediate **9** which is then deprotected to provide compound **10**. In Scheme 4, heterocycle **6** is coupled with a suitably substituted carboxylic acid to provide compound **11** which is deprotected as shown in Scheme 3. Compound **1** can be substituted for compound
10 **6** in both Schemes 3 and 4.

Scheme 5 outlines a generic synthesis of the macrocyclic compounds where R^{1d} is OH. The aldehyde **13** reacts with an organolithium or Grignard reagent to give the addition product **14** followed by oxidation to the ketone **15**. Addition of the requisite organolithium or Grignard to ketone **15** provides compound **16** which
15 then is oxidized to aldehyde **17**. Reductive alkylation of intermediate **5** with compound **17** provides compound **18**. Cyclization of **18** with cesium carbonate yields the macrocyclic compound **19** of the instant invention.

Scheme 6 illustrates the preparation of the macrocyclic compounds of the instant invention wherein W is imidazole and R^{1d} is NH₂. Imidazole **20** is
20 masked at the 2-position with TMSCl, then metallated a second time and reacted with aldehyde **13** to give compound **21**. Next, oxidation and treatment with t-butylsulfonamide provides compound **23**. Treatment of **23** with silyl-protected oxyalkyl Grignard reagent followed by removal of the silyl group gives alcohol **24**. Oxidation of the alcohol gives aldehyde **25** which reductively alkylates amine **5**, for
25 example, to give intermediate **26**. Cyclization with cesium carbonate provides the macrocycle compound **28** after deprotection.

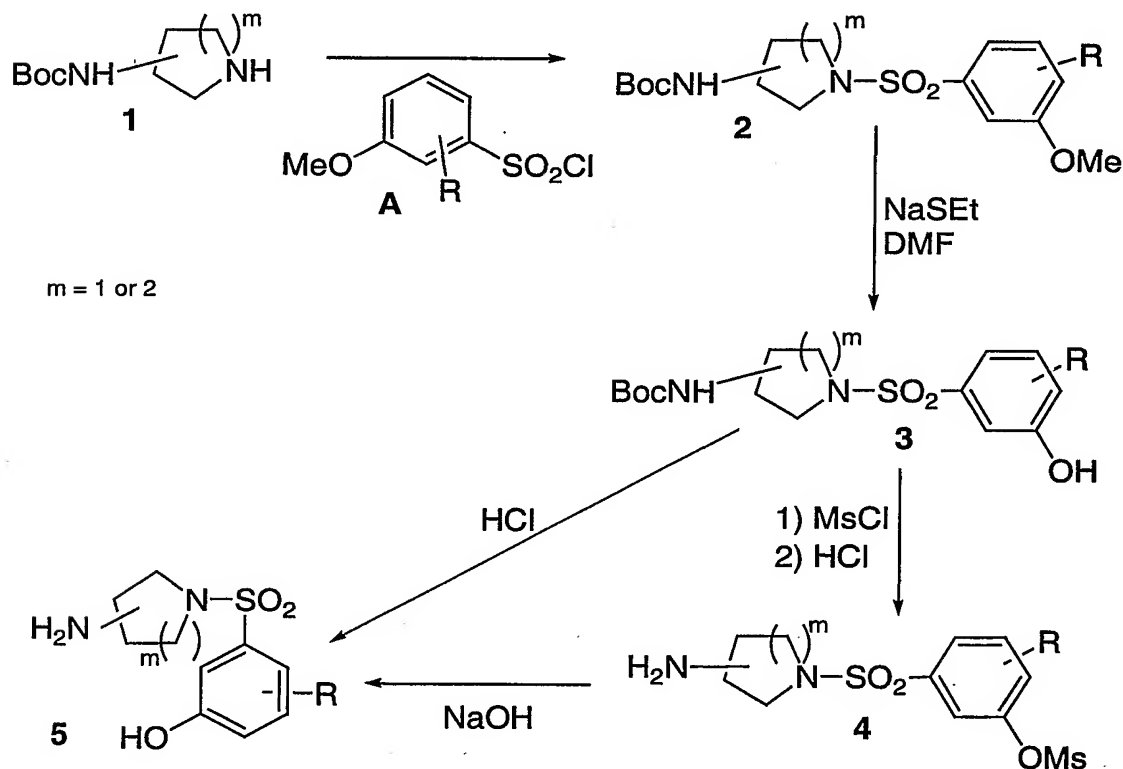
Scheme 7 shows an alternative method for the preparation of aldehyde **25**. Compound **23** is reacted with an alkenyl Grignard to give compound **29**. Subsequent oxidative cleavage of **29** provides aldehyde **25** which can be carried on as
30 illustrated in Scheme 6.

Scheme 8 describes the preparation of the macrocyclic compounds of the instant invention wherein the macrocycle is linked by an amide bond. Aldehyde **25** is oxidized to carboxylic acid **30** which is further coupled to compound **4**. Heating of aldehyde **25** with cesium carbonate removes the mesylate and forms a macrocyclic
35 compound of the instant invention **32**, after deprotection.

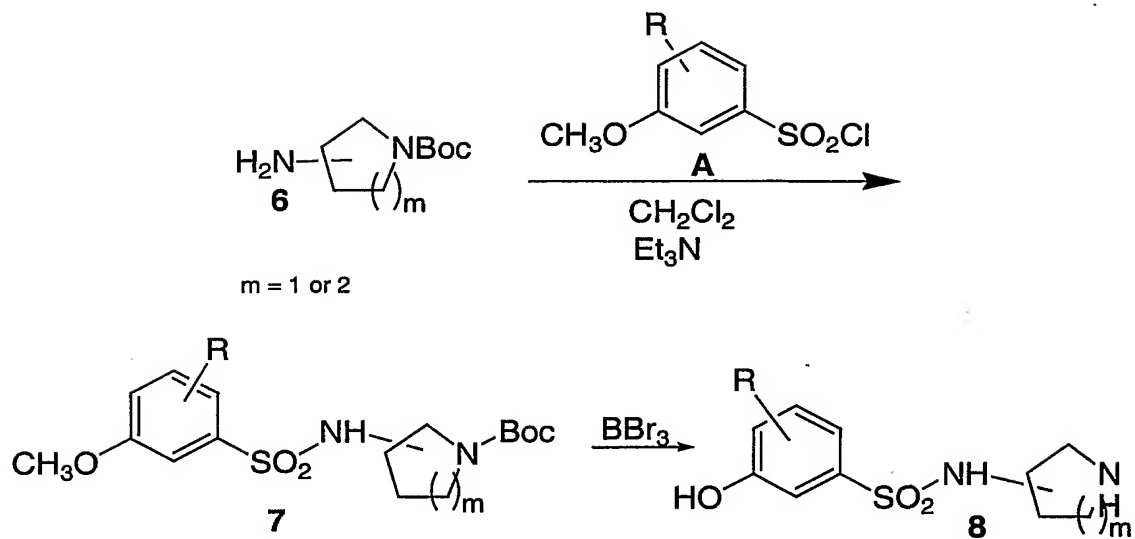
Scheme 9 illustrates macrocyclic compounds of the instant invention where the constraining heterocycle is an aminopyrrolidinone. The pyrrolidinone **36** was reductively alkylated with aldehyde **25** to give compound **37**. Cyclization of **37** with cesium carbonate followed by deprotection leads to the macrocyclic compound **39** of the instant invention.

In Scheme 10, heterocyclic carboxylic acids are prepared. Scheme 11 describes the macrocyclic compounds of the instant invention wherein the macrocyclic connection is through the heteroatom at the benzylic site between imidazole and fluorophenyl. Compound **43** was coupled to compounds **39** or **40** or **42** to give compound **44**. Removal of the methyl group with BBr_3 gives the phenol **45** which is cyclized to give the macrocyclic compounds **46** of the instant invention.

SCHEME 1

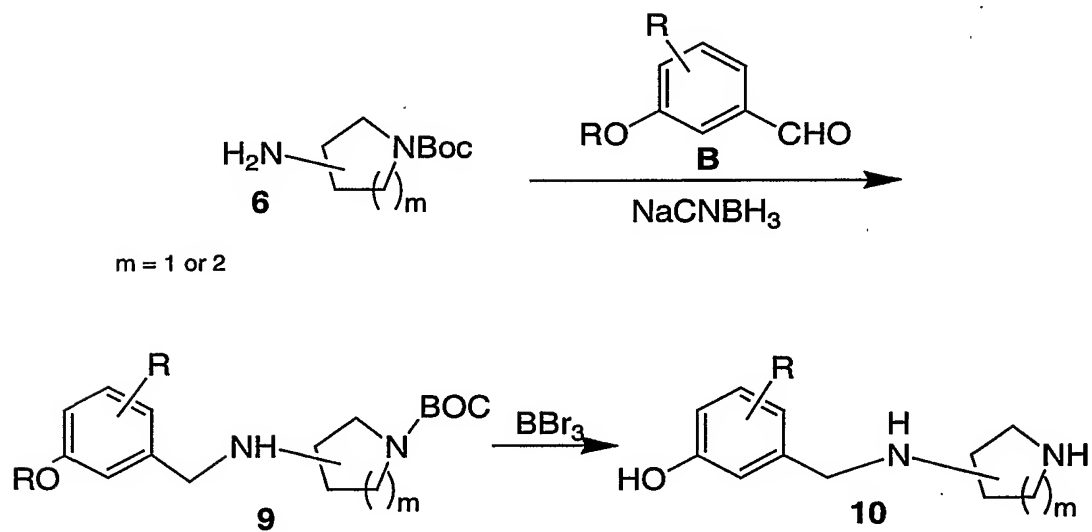


SCHEME 2

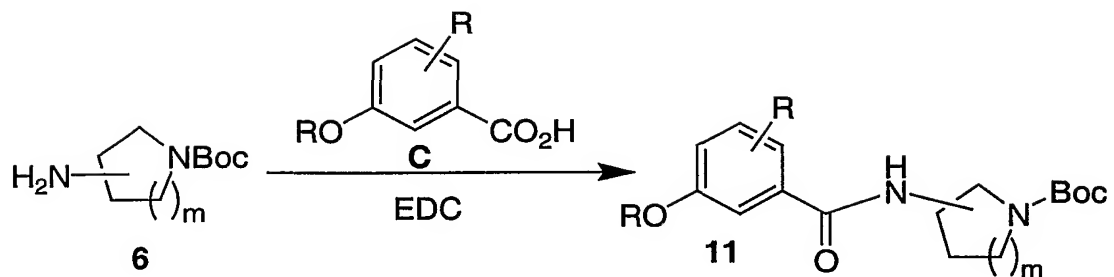


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SCHEME 3



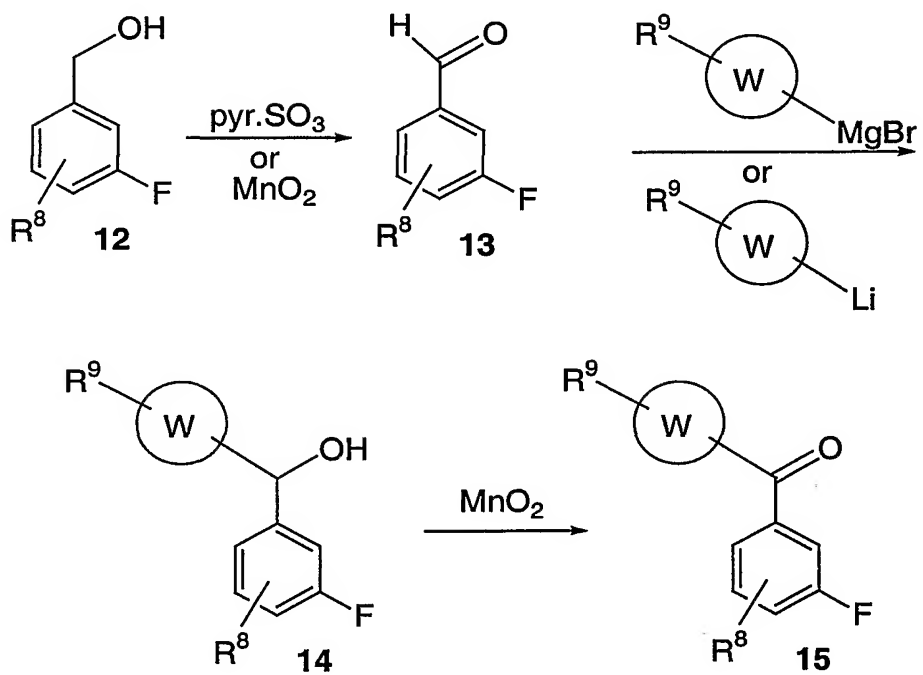
SCHEME 4



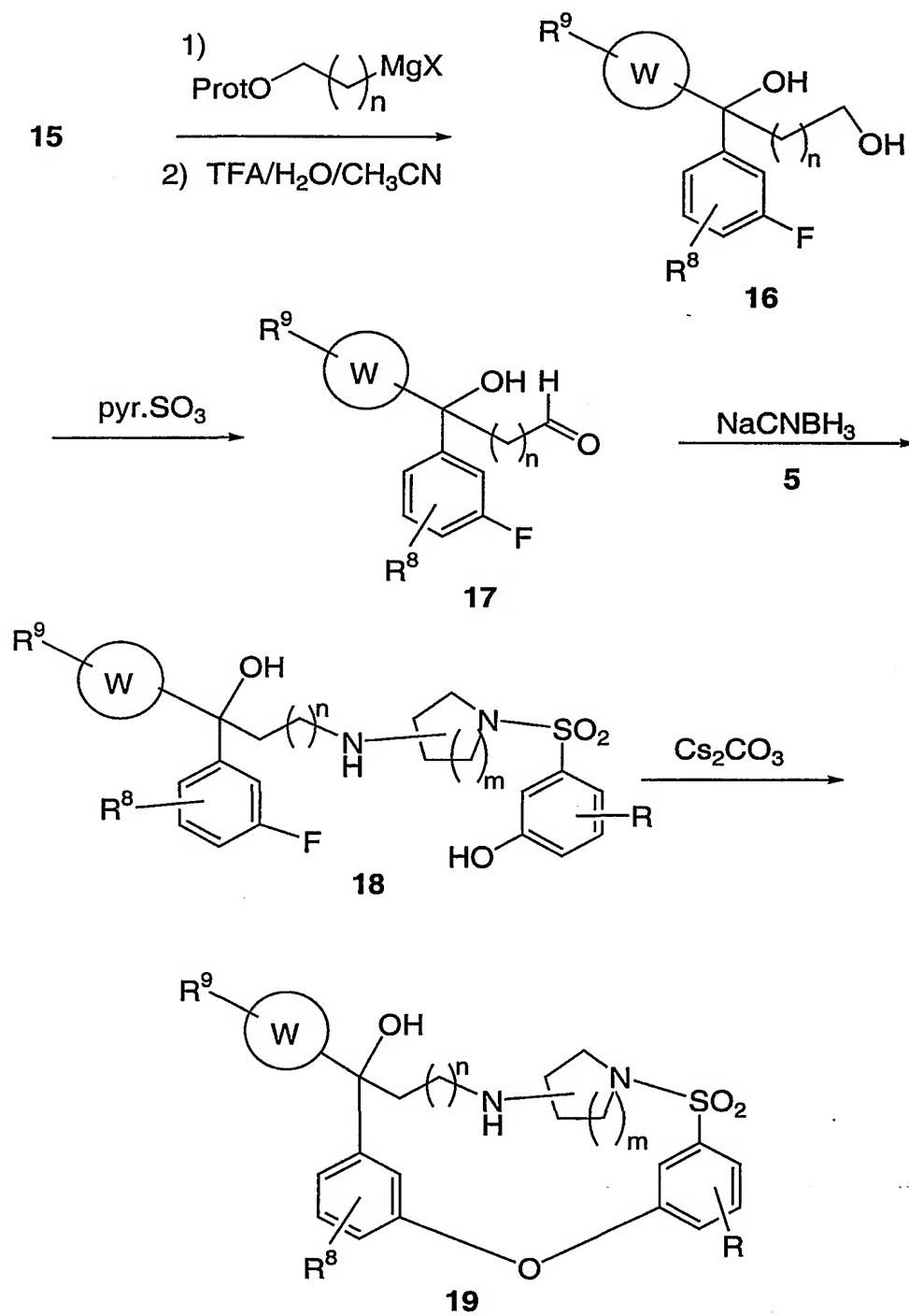
$m = 1 \text{ or } 2$

5

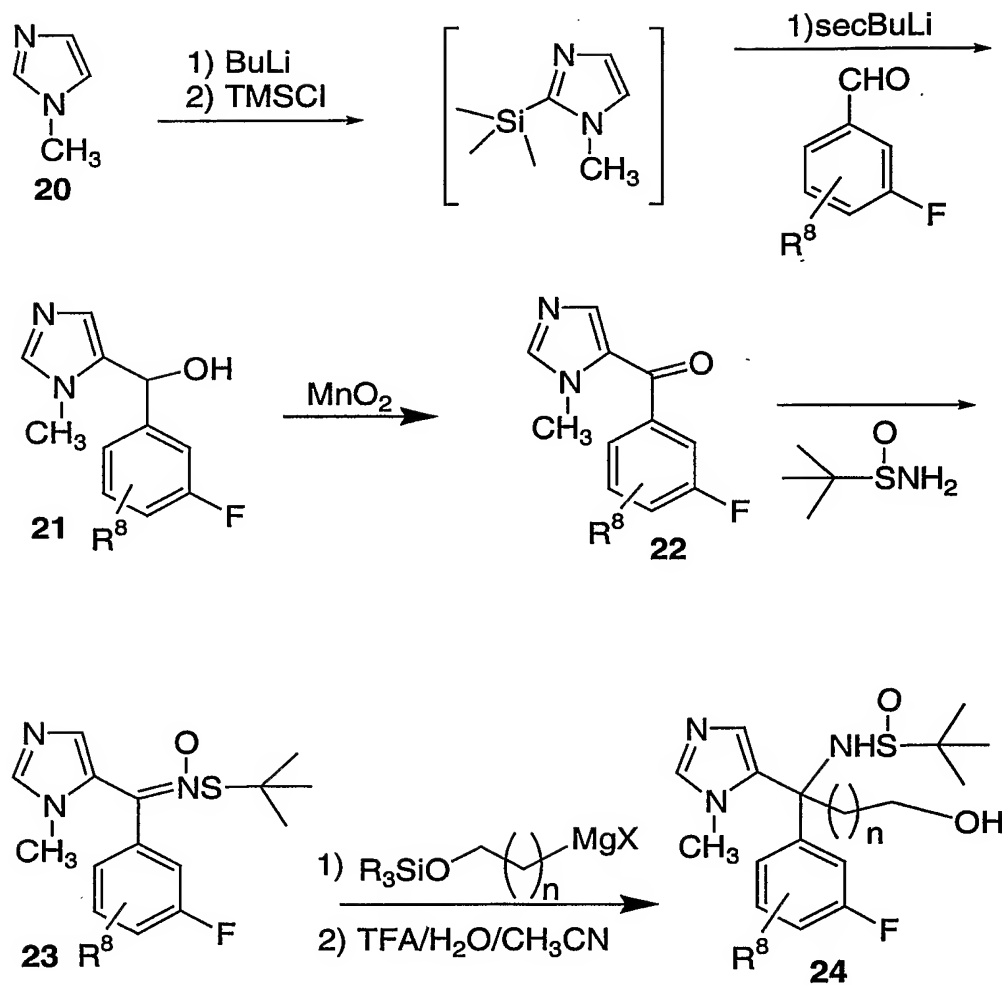
SCHEME 5



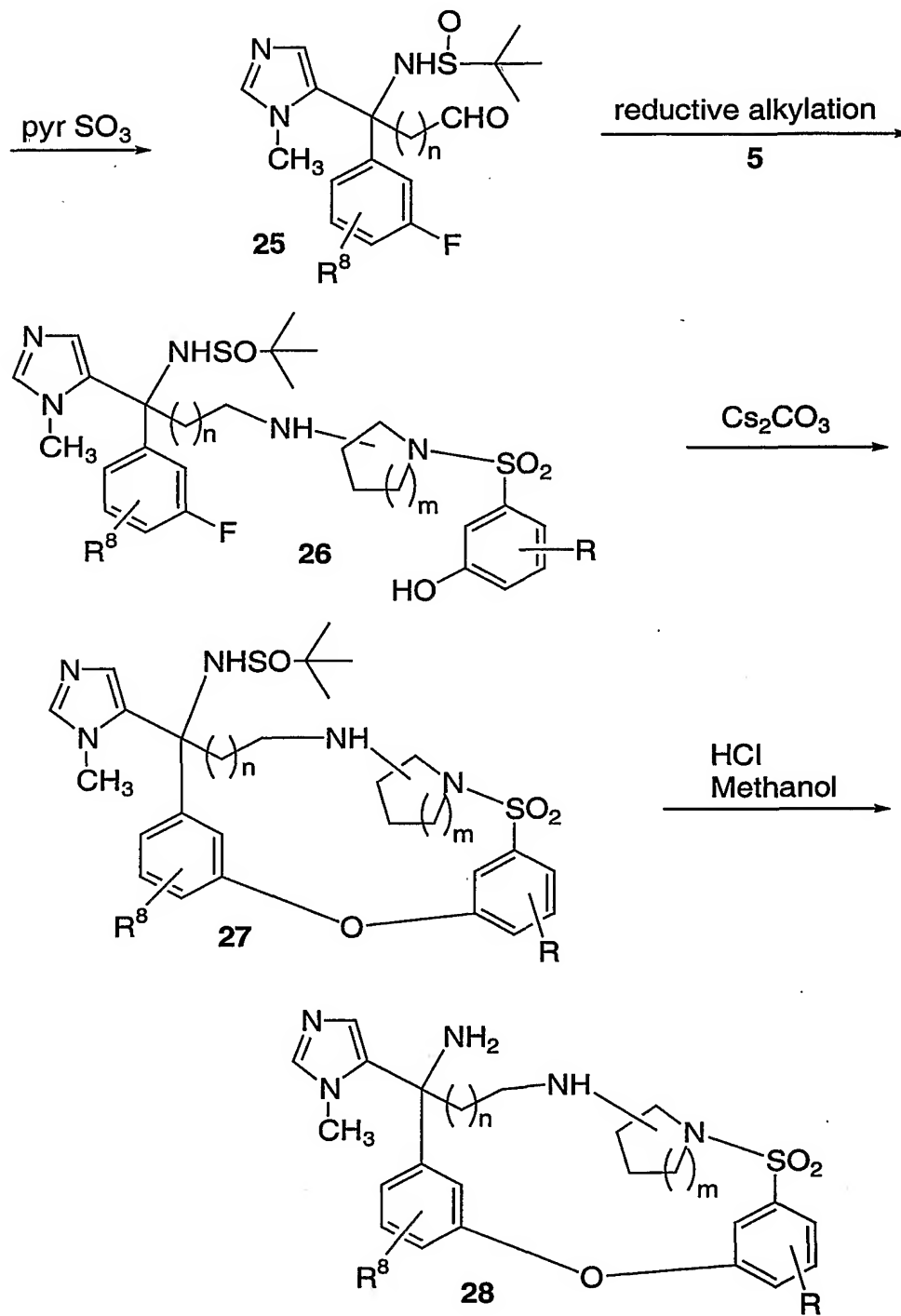
SCHEME 5 (CONTINUED)



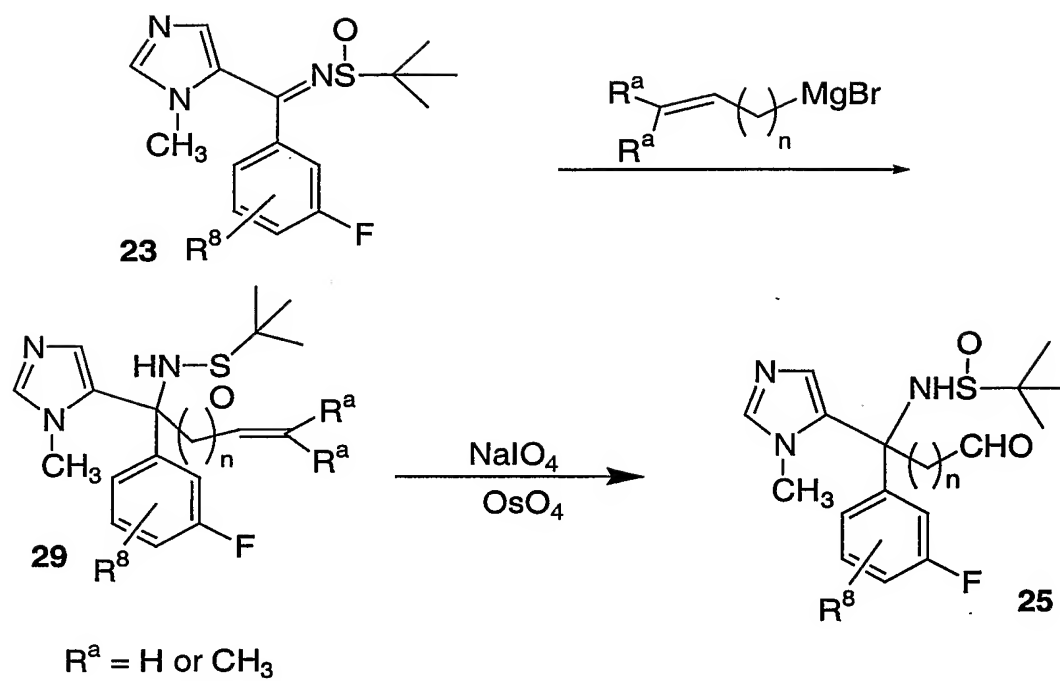
SCHEME 6



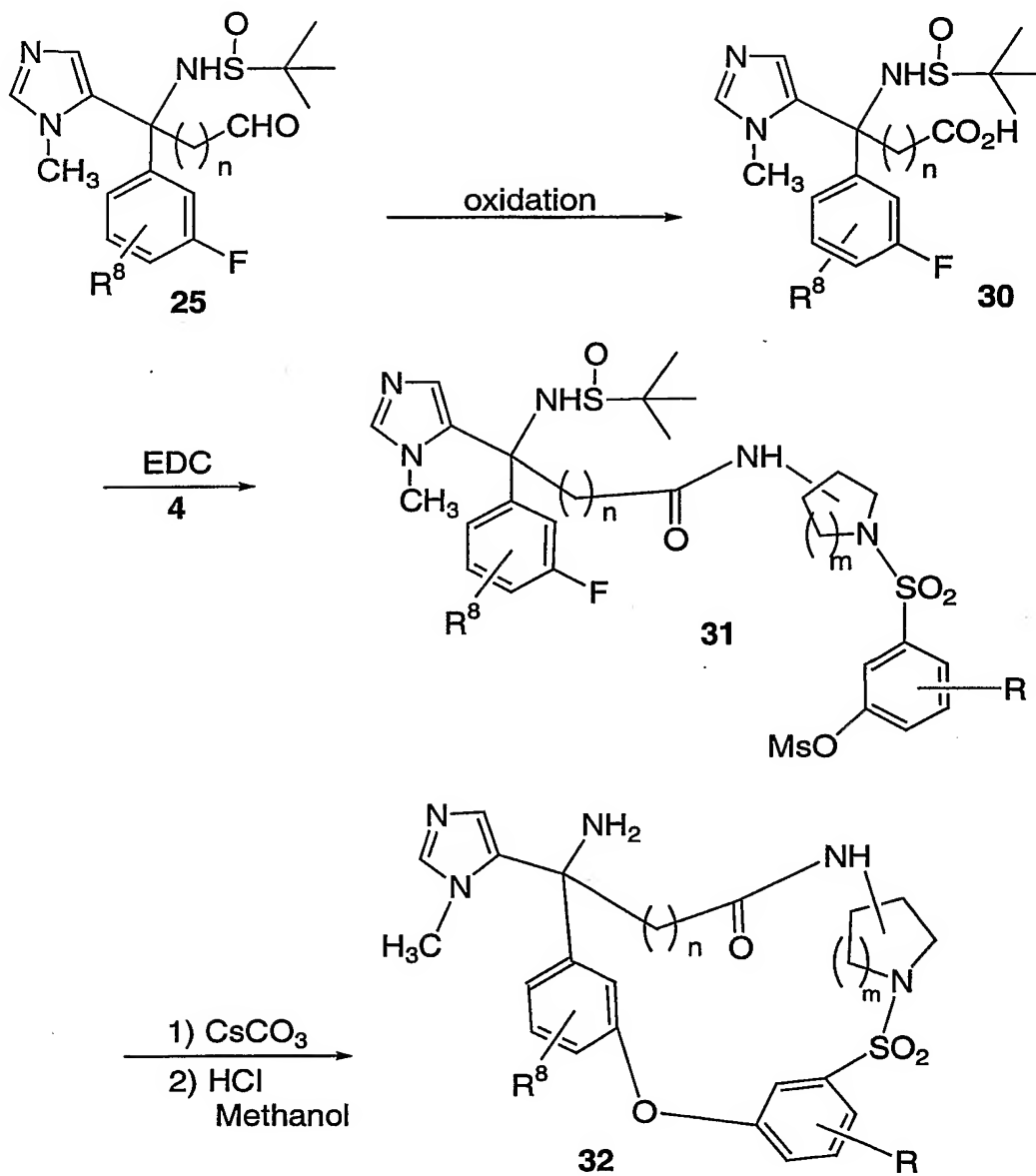
R is alkyl

SCHEME 6 (CONTINUED)

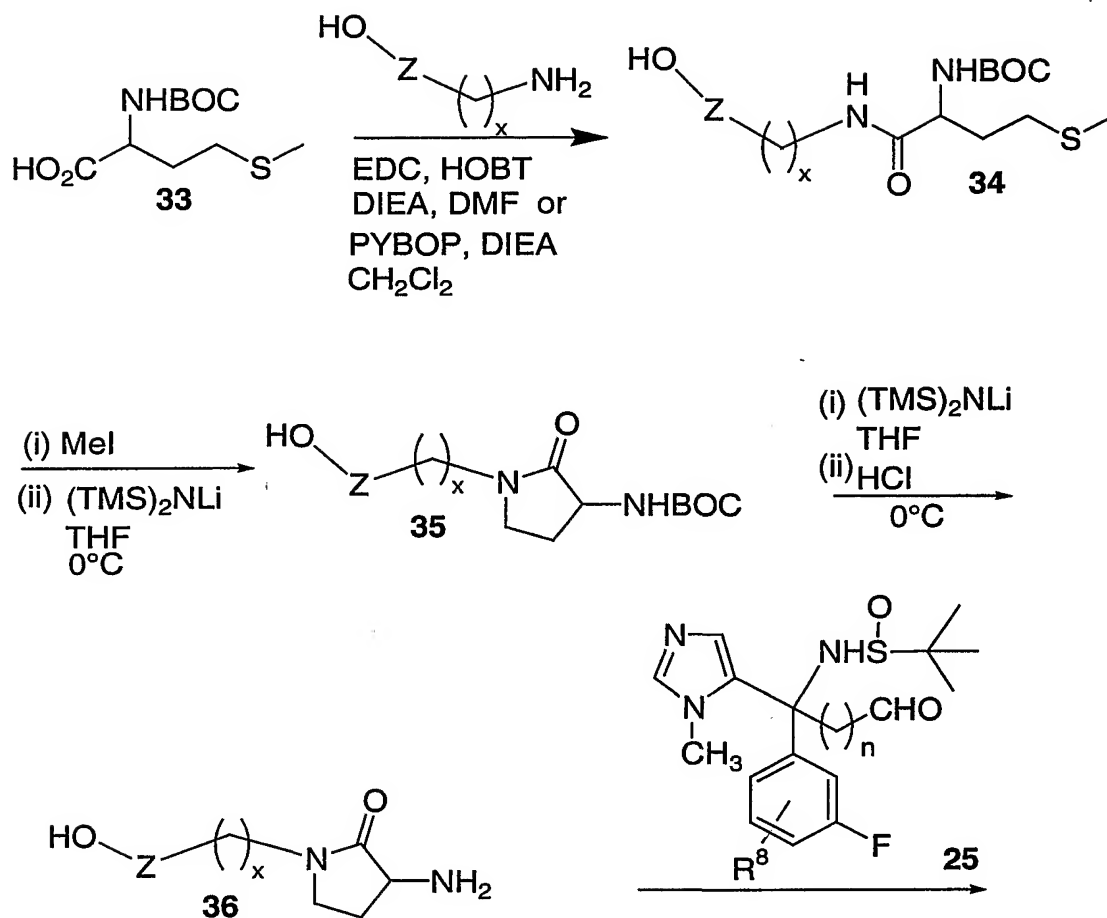
SCHEME 7



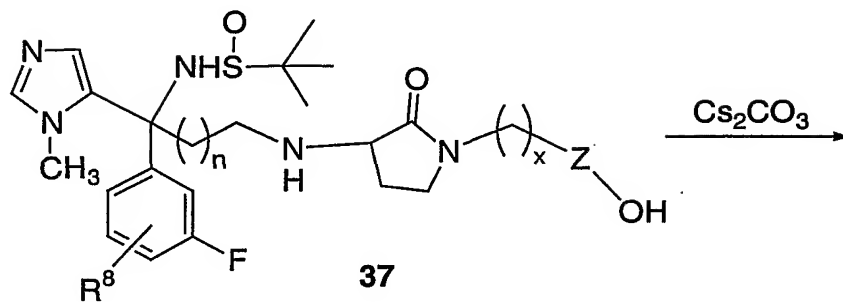
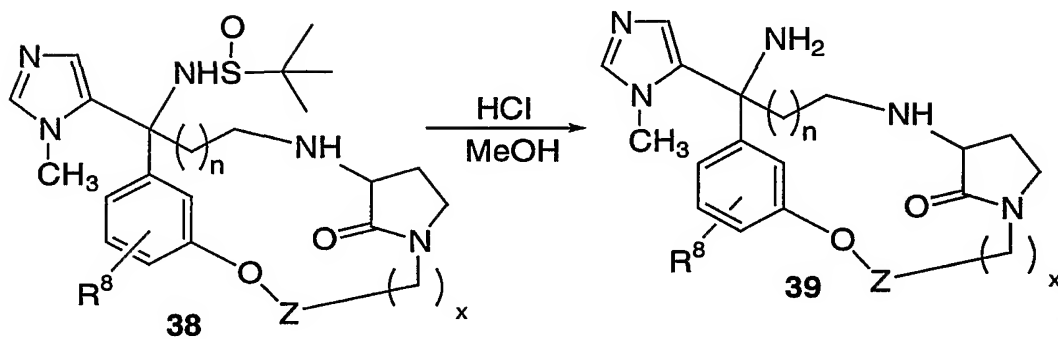
SCHEME 8



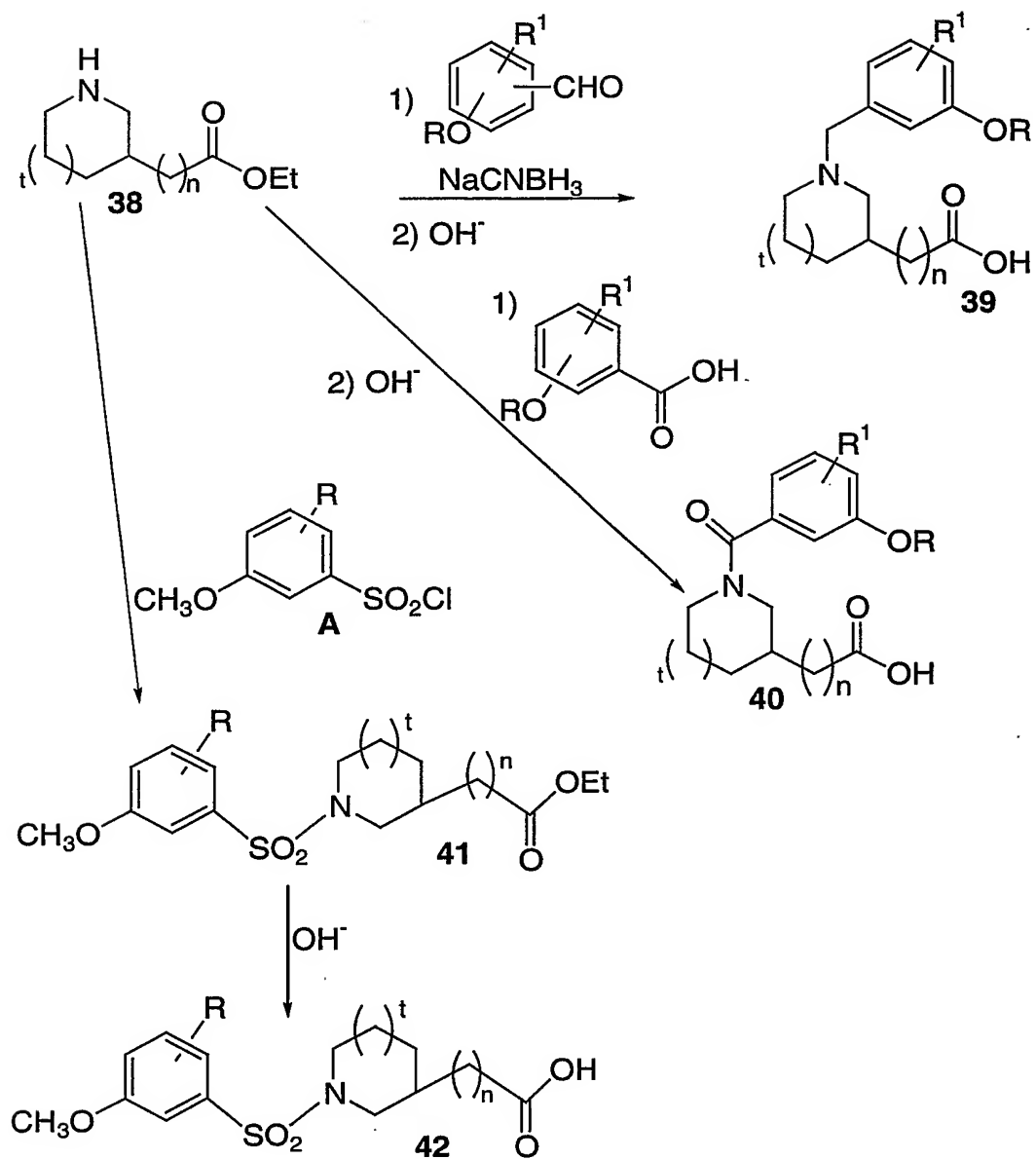
SCHEME 9



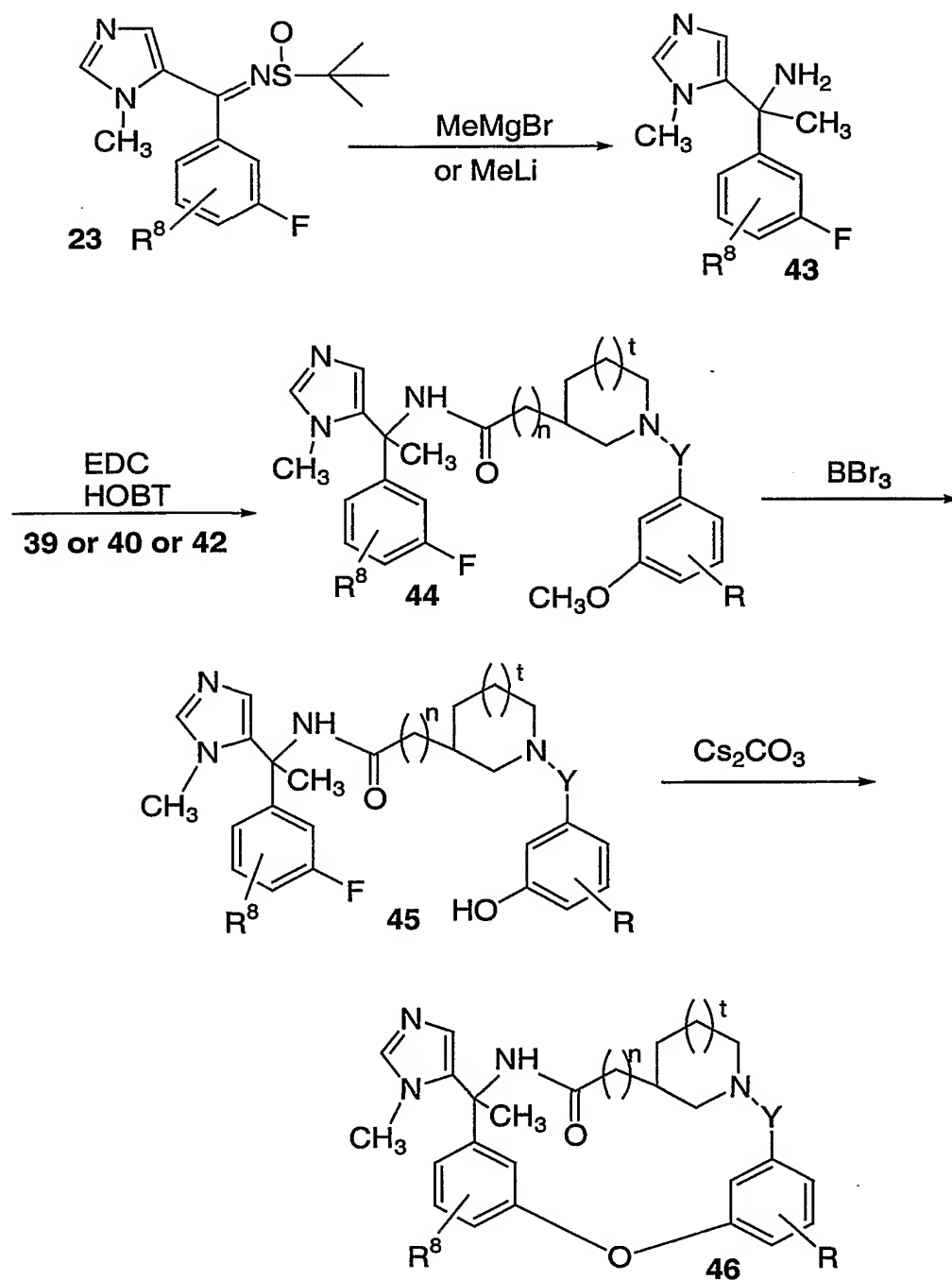
SCHEME 9 (CONTINUED)

 $x = 0 \text{ to } 6$  $x = 0 \text{ to } 6$

SCHEME 10



SCHEME 11



In a preferred embodiment of the instant invention the compounds of the invention are selective inhibitors of farnesyl-protein transferase. A compound is considered a selective inhibitor of farnesyl-protein transferase, for example, when its *in vitro* farnesyl-protein transferase inhibitory activity, as assessed by the assay described in Example 5, is at least 100 times greater than the *in vitro* activity of the same compound against geranylgeranyl-protein transferase-type I in the assay described in Example 6. Preferably, a selective compound exhibits at least 1000 times greater activity against one of the enzymatic activities when comparing geranylgeranyl-protein transferase-type I inhibition and farnesyl-protein transferase inhibition.

It is also preferred that the selective inhibitor of farnesyl-protein transferase is further characterized by:

- a) an IC₅₀ (a measure of *in vitro* inhibitory activity) for inhibition of the prenylation of newly synthesized K-Ras protein more than about 100-fold higher than the EC₅₀ for the inhibition of the farnesylation of hDJ protein. When measuring such IC₅₀s and EC₅₀s the assays described in Example 10 may be utilized.

It is also preferred that the selective inhibitor of farnesyl-protein transferase is further characterized by:

- b) an IC₅₀ (a measurement of *in vitro* inhibitory activity) for inhibition of K4B-Ras dependent activation of MAP kinases in cells at least 100-fold greater than the EC₅₀ for inhibition of the farnesylation of the protein hDJ in cells.

It is also preferred that the selective inhibitor of farnesyl-protein transferase is further characterized by:

- c) an IC₅₀ (a measurement of *in vitro* inhibitory activity) against H-Ras dependent activation of MAP kinases in cells at least 1000 fold lower than the inhibitory activity (IC₅₀) against H-ras-CVLL (SEQ.ID.NO.: 1) dependent activation of MAP kinases in cells.

When measuring Ras dependent activation of MAP kinases in cells the assays described in Example 9 may be utilized.

In another preferred embodiment of the instant invention the compounds of the invention are dual inhibitors of farnesyl-protein transferase and geranylgeranyl-protein transferase type I. Such a dual inhibitor may be termed a

Class II prenyl-protein transferase inhibitor and will exhibit certain characteristics when assessed in *in vitro* assays, which are dependent on the type of assay employed.

In a SEAP assay, such as described in Example 9, it is preferred that the dual inhibitor compound has an *in vitro* inhibitory activity (IC₅₀) that is less
5 than about 12μM against K4B-Ras dependent activation of MAP kinases in cells.

The Class II prenyl-protein transferase inhibitor may also be characterized by:

- a) an IC₅₀ (a measurement of *in vitro* inhibitory activity) for inhibiting K4B-Ras dependent activation of MAP kinases in cells between 0.1 and 100
10 times the IC₅₀ for inhibiting the farnesylation of the protein hDJ in cells; and
- b) an IC₅₀ (a measurement of *in vitro* inhibitory activity) for inhibiting K4B-Ras dependent activation of MAP kinases in cells greater than 5-fold lower than the inhibitory activity (IC₅₀) against expression of the SEAP
15 protein in cells transfected with the pCMV-SEAP plasmid that constitutively expresses the SEAP protein.

The Class II prenyl-protein transferase inhibitor may also be characterized by:

- a) an IC₅₀ (a measurement of *in vitro* inhibitory activity) against H-Ras dependent activation of MAP kinases in cells greater than 2 fold lower but
20 less than 20,000 fold lower than the inhibitory activity (IC₅₀) against H-ras-CVLL (SEQ.ID.NO.: 1) dependent activation of MAP kinases in cells; and
- b) an IC₅₀ (a measurement of *in vitro* inhibitory activity) against H-ras-CVLL dependent activation of MAP kinases in cells greater than 5-fold lower
25 than the inhibitory activity (IC₅₀) against expression of the SEAP protein in cells transfected with the pCMV-SEAP plasmid that constitutively expresses the SEAP protein.

The Class II prenyl-protein transferase inhibitor may also be characterized by:

- a) an IC₅₀ (a measurement of *in vitro* inhibitory activity) against H-Ras dependent activation of MAP kinases in cells greater than 10-fold lower but
30 less than 2,500 fold lower than the inhibitory activity (IC₅₀) against H-ras-CVLL (SEQ.ID.NO.: 1) dependent activation of MAP kinases in cells; and
- b) an IC₅₀ (a measurement of *in vitro* inhibitory activity) against H-ras-CVLL dependent activation of MAP kinases in cells greater than 5 fold lower
35

than the inhibitory activity (IC₅₀) against expression of the SEAP protein in cells transfected with the pCMV-SEAP plasmid that constitutively expresses the SEAP protein.

5 A method for measuring the activity of the inhibitors of prenyl-protein transferase, as well as the instant combination compositions, utilized in the instant methods against Ras dependent activation of MAP kinases in cells is described in Example 9.

10 In yet another embodiment, a compound of the instant invention may be a more potent inhibitor of geranylgeranyl-protein transferase-type I than it is an inhibitor of farnesyl-protein transferase.

The instant compounds are useful as pharmaceutical agents for mammals, especially for humans. These compounds may be administered to patients for use in the treatment of cancer. Examples of the type of cancer which may be treated with the compounds of this invention include, but are not limited to, colorectal carcinoma, exocrine pancreatic carcinoma, myeloid leukemias and neurological tumors. Such tumors may arise by mutations in the ras genes themselves, mutations in the proteins that can regulate Ras activity (i.e., neurofibromin (NF-1), neu, src, abl, lck, fyn) or by other mechanisms.

20 The compounds of the instant invention inhibit farnesyl-protein transferase and the farnesylation of the oncogene protein Ras. The instant compounds may also inhibit tumor angiogenesis, thereby affecting the growth of tumors (J. Rak et al. Cancer Research, 55:4575-4580 (1995)). Such anti-angiogenesis properties of the instant compounds may also be useful in the treatment of certain forms of vision deficit related to retinal vascularization.

25 The compounds of this invention are also useful for inhibiting other proliferative diseases, both benign and malignant, wherein Ras proteins are aberrantly activated as a result of oncogenic mutation in other genes (i.e., the Ras gene itself is not activated by mutation to an oncogenic form) with said inhibition being accomplished by the administration of an effective amount of the compounds of the invention to a mammal in need of such treatment. For example, the composition is useful in the treatment of neurofibromatosis, which is a benign proliferative disorder.

30 The instant compounds may also be useful in the treatment of certain viral infections, in particular in the treatment of hepatitis delta and related viruses (J.S. Glenn et al. Science, 256:1331-1333 (1992)).

The compounds of the instant invention are also useful in the prevention of restenosis after percutaneous transluminal coronary angioplasty by inhibiting neointimal formation (C. Indolfi et al. *Nature medicine*, 1:541-545(1995)).

5 The instant compounds may also be useful in the treatment and prevention of polycystic kidney disease (D.L. Schaffner et al. *American Journal of Pathology*, 142:1051-1060 (1993) and B. Cowley, Jr. et al., *FASEB Journal*, 2:A3160 (1988)).

The instant compounds may also be useful for the treatment of fungal infections.

10 The instant compounds may also be useful as inhibitors of proliferation of vascular smooth muscle cells and therefore useful in the prevention and therapy of arteriosclerosis and diabetic vascular pathologies.

The compounds of the instant invention may also be useful in the prevention and treatment of endometriosis, uterine fibroids, dysfunctional uterine bleeding and endometrial hyperplasia.

15 In such methods of prevention and treatment as described herein, the prenyl-protein transferase inhibitors of the instant invention may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated. For example, the prenyl-protein transferase inhibitor may be useful in further combination with drugs known to suppress the activity of the ovaries and slow the growth of the endometrial tissue. Such drugs include but are not limited to oral contraceptives, progestins, danazol and GnRH (gonadotropin-releasing hormone) agonists.

20 Administration of the prenyl-protein transferase inhibitor may also be combined with surgical treatment of endometriosis (such as surgical removal of misplaced endometrial tissue) where appropriate.

The instant compounds may also be useful as inhibitors of corneal inflammation. These compounds may improve the treatment of corneal opacity which results from cauterization-induced corneal inflammation. The instant compounds may also be useful in reducing corneal edema and neovascularization. (K. Sonoda et al., *Invest. Ophthalmol. Vis. Sci.*, 1998, vol. 39, p 2245-2251).

30 The compounds of this invention may be administered to mammals, preferably humans, either alone or, preferably, in combination with pharmaceutically acceptable carriers, excipients or diluents, in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or

parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

Additionally, the compounds of the instant invention may be administered to a mammal in need thereof using a gel extrusion mechanism (GEM) device, such as that described in US Serial No. 60/144,643, filed on July 20, 1999, which is hereby incorporated by reference.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specific amounts, as well as any product which results, directly or indirectly, from combination of the specific ingredients in the specified amounts.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, microcrystalline cellulose, sodium crosscarmellose, corn starch, or alginic acid; binding agents, for example starch, gelatin, polyvinyl-pyrrolidone or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to mask the unpleasant taste of the drug or delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a water soluble taste masking material such as hydroxypropyl-methylcellulose or hydroxypropyl-cellulose, or a time delay material such as ethyl cellulose, cellulose acetate butyrate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water soluble carrier such as polyethyl-

eneglycol or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxyctanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as butylated hydroxyanisol or alpha-tocopherol.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

The pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phospho-

tides, for example soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavoring agents, 5 preservatives and antioxidants.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, flavoring and coloring agents and antioxidant.

The pharmaceutical compositions may be in the form of a sterile 10 injectable aqueous solutions. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution.

The sterile injectable preparation may also be a sterile injectable oil-in-water microemulsion where the active ingredient is dissolved in the oily phase. For example, the active ingredient may be first dissolved in a mixture of soybean oil 15 and lecithin. The oil solution then introduced into a water and glycerol mixture and processed to form a microemulsion.

The injectable solutions or microemulsions may be introduced into a patient's blood-stream by local bolus injection. Alternatively, it may be advantageous to administer the solution or microemulsion in such a way as to maintain a constant 20 circulating concentration of the instant compound. In order to maintain such a constant concentration, a continuous intravenous delivery device may be utilized. An example of such a device is the Deltec CADD-PLUS™ model 5400 intravenous pump.

The pharmaceutical compositions may be in the form of a sterile 25 injectable aqueous or oleagenous suspension for intramuscular and subcutaneous administration. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for 30 example as a solution in 1,3-butane diol. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of Formula A may also be administered in the form of

a suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compound of Formula A are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

The compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles and delivery devices, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen. Compounds of the present invention may also be delivered as a suppository employing bases such as cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

When a compound according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, sex and response of the individual patient, as well as the severity of the patient's symptoms.

In one exemplary application, a suitable amount of compound is administered to a mammal undergoing treatment for cancer. Administration occurs in an amount between about 0.1 mg/kg of body weight to about 60 mg/kg of body weight per day, preferably of between 0.5 mg/kg of body weight to about 40 mg/kg of body weight per day.

The compounds of the instant invention may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated. For example, the compounds of the instant invention may also be co-administered with other well known cancer therapeutic agents that are selected for their particular usefulness against the condition that is being treated. Included in such combinations of therapeutic agents are combinations of the instant prenyl-protein transferase inhibitors and an antineoplastic agent.

It is also understood that such a combination of antineoplastic agent and inhibitor of prenyl-protein transferase may be used in conjunction with other methods of treating cancer and/or tumors, including radiation therapy and surgery. It is further understood that any of the therapeutic agents described herein may also be used in
5 combination with a compound of the instant invention and an antineoplastic agent.

Examples of an antineoplastic agent include, in general, microtubule-stabilizing agents such as paclitaxel (also known as Taxol®), docetaxel (also known as Taxotere®), epothilone A, epothilone B, desoxyepothilone A, desoxyepothilone B or their derivatives); microtubule-disruptor agents; alkylating agents, for example,
10 nitrogen mustards, ethyleneimine compounds, alkyl sulfonates and other compounds with an alkylating action such as nitrosoureas, cisplatin, and dacarbazine; anti-metabolites, for example, folic acid, purine or pyrimidine antagonists; epidophyllotoxin; an antineoplastic enzyme; a topoisomerase inhibitor; procarbazine; mitoxantrone; platinum coordination complexes; biological response modifiers and
15 growth inhibitors; mitotic inhibitors, for example, vinca alkaloids and derivatives of podophyllotoxin; cytotoxic antibiotics; hormonal/anti-hormonal therapeutic agents, haematopoietic growth factors and antibodies (such as trastuzumab, also known as Herceptin™).

Example classes of antineoplastic agents include, for example, the
20 anthracycline family of drugs, the vinca drugs, the mitomycins, the bleomycins, the cytotoxic nucleosides, the taxanes, the epothilones, discodermolide, the pteridine family of drugs, diynenes and the podophyllotoxins. Particularly useful members of those classes include, for example, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, dichloro-methotrexate, mitomycin C,
25 porfiromycin, 5-fluorouracil, 6-mercaptopurine, gemcitabine, cytosine arabinoside, podophyllotoxin or podo-phyllotoxin derivatives such as etoposide, etoposide phosphate or teniposide, melphalan, vinblastine, vincristine, leurosine, vindesine, leurosine, paclitaxel and the like. Other useful antineoplastic agents include
estramustine, cisplatin, carboplatin, cyclophosphamide, bleomycin, tamoxifen,
30 ifosamide, melphalan, hexamethyl melamine, thiotepa, cytarabin, idatrexate, trimetrexate, dacarbazine, L-asparaginase, dactinomycin, mechlorethamine (nitrogen mustard), streptozocin, cyclophosphamide, carmustine (BCNU), lomustine (CCNU), procarbazine, mitomycin, cytarabine, etoposide, methotrexate, bleomycin, chlorambucil, camptothecin, CPT-11, topotecan, ara-C, bicalutamide, flutamide,
35 leuprolide, pyridobenzoindole derivatives, interferons and interleukins. Particular

examples of antineoplastic, or chemotherapeutic, agents are described, for example, by D. J. Stewart in "Nausea and Vomiting: Recent Research and Clinical Advances", Eds. J. Kucharczyk, et al., CRC Press Inc., Boca Raton, Florida, USA (1991), pages 177-203, especially page 188. See also, R. J. Gralla, et al., Cancer Treatment
5 Reports, 68(1), 163-172 (1984).

The preferred class of antineoplastic agents is the taxanes and the preferred antineoplastic agent is paclitaxel.

The compounds of the instant invention may also be co-administered with antisense oligonucleotides which are specifically hybridizable with RNA or
10 DNA deriving from human *ras* gene. Such antisense oligonucleotides are described in U.S. Patent. No. 5,576,208 and PCT Publication No. WO 99/22772. The instant compounds are particularly useful when co-administered with the antisense oligonucleotide comprising the amino acid sequence of SEQ.ID.NO: 2 of U.S. Patent No. 5,576,208.

15 Certain compounds of the instant invention may exhibit very low plasma concentrations and significant inter-individual variation in the plasma levels of the compound. It is believed that very low plasma concentrations and high inter-subject variability achieved following administration of certain prenyl-protein transferase inhibitors to mammals may be due to extensive metabolism by cytochrome
20 P450 enzymes prior to entry of drug into the systemic circulation. Prenyl-protein transferase inhibitors may be metabolized by cytochrome P450 enzyme systems, such as CYP3A4, CYP2D6, CYP2C9, CYP2C19 or other cytochrome P450 isoform. If a compound of the instant invention demonstrates an affinity for one or more of the cytochrome P450 enzyme systems, another compound with a higher affinity for the
25 P450 enzyme(s) involved in metabolism should be administered concomitantly. Examples of compounds that have a comparatively very high affinity for CYP3A4, CYP2D6, CYP2C9, CYP2C19 or other P450 isoform include, but are not limited to, piperonyl butoxide, troleandomycin, erythromycin, proadifen, isoniazid, allyliso-propylacetamide, ethinylestradiol, chloramphenicol, 2-ethynylnaphthalene and the
30 like. Such a high affinity compound, when employed in combination with a compound of formula A, may reduce the inter-individual variation and increase the plasma concentration of a compound of formula A to a level having substantial therapeutic activity by inhibiting the metabolism of the compound of formula A. Additionally, inhibiting the metabolism of a compound of the instant invention
35 prolongs the pharmacokinetic half-life, and thus the pharmacodynamic effect,

of the compound.

A compound of the present invention may be employed in conjunction with antiemetic agents to treat nausea or emesis, including acute, delayed, late-phase, and anticipatory emesis, which may result from the use of a compound of the present invention, alone or with radiation therapy. For the prevention or treatment of emesis a compound of the present invention may be used in conjunction with other anti-emetic agents, especially neurokinin-1 receptor antagonists, 5HT3 receptor antagonists, such as ondansetron, granisetron, tropisetron, and zatisetron, GABAB receptor agonists, such as baclofen, or a corticosteroid such as Decadron (dexamethasone), Kenalog, Aristocort, Nasalide, Preferid, Benecorten or others such as disclosed in U.S. Patent Nos. 2,789,118, 2,990,401, 3,048,581, 3,126,375, 3,929,768, 3,996,359, 3,928,326 and 3,749,712. For the treatment or prevention of emesis, conjunctive therapy with a neurokinin-1 receptor antagonist, a 5HT3 receptor antagonist and a corticosteroid is preferred.

Neurokinin-1 receptor antagonists of use in conjunction with the compounds of the present invention are fully described, for example, in U.S. Patent Nos. 5,162,339, 5,232,929, 5,242,930, 5,373,003, 5,387,595, 5,459,270, 5,494,926, 5,496,833, 5,637,699, 5,719,147; European Patent Publication Nos. EP 0 360 390, 0 394 989, 0 428 434, 0 429 366, 0 430 771, 0 436 334, 0 443 132, 0 482 539, 0 498 069, 0 499 313, 0 512 901, 0 512 902, 0 514 273, 0 514 274, 0 514 275, 0 514 276, 0 515 681, 0 517 589, 0 520 555, 0 522 808, 0 528 495, 0 532 456, 0 533 280, 0 536 817, 0 545 478, 0 558 156, 0 577 394, 0 585 913, 0 590 152, 0 599 538, 0 610 793, 0 634 402, 0 686 629, 0 693 489, 0 694 535, 0 699 655, 0 699 674, 0 707 006, 0 708 101, 0 709 375, 0 709 376, 0 714 891, 0 723 959, 0 733 632 and 0 776 893; PCT International Patent Publication Nos. WO 90/05525, 90/05729, 91/09844, 91/18899, 92/01688, 92/06079, 92/12151, 92/15585, 92/17449, 92/20661, 92/20676, 92/21677, 92/22569, 93/00330, 93/00331, 93/01159, 93/01165, 93/01169, 93/01170, 93/06099, 93/09116, 93/10073, 93/14084, 93/14113, 93/18023, 93/19064, 93/21155, 93/21181, 93/23380, 93/24465, 94/00440, 94/01402, 94/02461, 94/02595, 94/03429, 94/03445, 94/04494, 94/04496, 94/05625, 94/07843, 94/08997, 94/10165, 94/10167, 94/10168, 94/10170, 94/11368, 94/13639, 94/13663, 94/14767, 94/15903, 94/19320, 94/19323, 94/20500, 94/26735, 94/26740, 94/29309, 95/02595, 95/04040, 95/04042, 95/06645, 95/07886, 95/07908, 95/08549, 95/11880, 95/14017, 95/15311, 95/16679, 95/17382, 95/18124, 95/18129, 95/19344, 95/20575, 95/21819, 95/22525, 95/23798, 95/26338, 95/28418, 95/30674, 95/30687, 95/33744, 96/05181,

96/05193, 96/05203, 96/06094, 96/07649, 96/10562, 96/16939, 96/18643, 96/20197, 96/21661, 96/29304, 96/29317, 96/29326, 96/29328, 96/31214, 96/32385, 96/37489, 97/01553, 97/01554, 97/03066, 97/08144, 97/14671, 97/17362, 97/18206, 97/19084, 97/19942 and 97/21702; and in British Patent Publication Nos. 2 266 529, 2 268 931, 2 269 170, 2 269 590, 2 271 774, 2 292 144, 2 293 168, 2 293 169, and 2 302 689. The preparation of such compounds is fully described in the aforementioned patents and publications.

A particularly preferred neurokinin-1 receptor antagonist for use in conjunction with the compounds of the present invention is 2-(R)-(1-(R)-(3,5-bis (trifluoromethyl)phenyl)ethoxy)-3-(S)-(4-fluorophenyl)-4-(3-(5-oxo-1H,4H-1,2,4-triazolo)methyl)morpholine, or a pharmaceutically acceptable salt thereof, which is described in U.S. Patent No. 5,719,147.

For the treatment of cancer, it may be desirable to employ a compound of the present invention in conjunction with another pharmacologically active agent(s). A compound of the present invention and the other pharmacologically active agent(s) may be administered to a patient simultaneously, sequentially or in combination. For example, the present compound may employed directly in combination with the other active agent(s), or it may be administered prior, concurrent or subsequent to the administration of the other active agent(s). In general, the currently available dosage forms of the known therapeutic agents for use in such combinations will be suitable.

For example, a compound of the present invention may be presented together with another therapeutic agent in a combined preparation, such as with an antiemetic agent for simultaneous, separate, or sequential use in the relief of emesis associated with employing a compound of the present invention and radiation therapy. Such combined preparations may be, for example, in the form of a twin pack. A preferred combination comprises a compound of the present invention with antiemetic agents, as described above.

Radiation therapy, including x-rays or gamma rays which are delivered from either an externally applied beam or by implantation of tiny radioactive sources, may also be used in combination with the instant inhibitor of prenyl-protein transferase alone to treat cancer.

Additionally, compounds of the instant invention may also be useful as radiation sensitizers, as described in WO 97/38697, published on October 23, 1997, and herein incorporated by reference.

The instant compounds may also be useful in combination with other inhibitors of parts of the signaling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Thus, the instant compounds may be utilized in combination with farnesyl pyrophosphate competitive inhibitors of the activity of farnesyl-protein transferase or in combination with a compound which has Raf antagonist activity. The instant compounds may also be co-administered with compounds that are selective inhibitors of geranylgeranyl protein transferase.

In particular, if the compound of the instant invention is a selective inhibitor of farnesyl-protein transferase, co-administration with a compound(s) that is a selective inhibitor of geranylgeranyl protein transferase may provide an improved therapeutic effect.

In particular, the compounds disclosed in the following patents and publications may be useful as farnesyl pyrophosphate-competitive inhibitor component of the instant composition: U.S. Serial Nos. 08/254,228 and 08/435,047. Those patents and publications are incorporated herein by reference.

In practicing methods of this invention, which comprise administering, simultaneously or sequentially or in any order, two or more of a protein substrate-competitive inhibitor and a farnesyl pyrophosphate-competitive inhibitor, such administration can be orally or parenterally, including intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration. It is preferred that such administration be orally. It is more preferred that such administration be orally and simultaneously. When the protein substrate-competitive inhibitor and farnesyl pyrophosphate-competitive inhibitor are administered sequentially, the administration of each can be by the same method or by different methods.

The instant compounds may also be useful in combination with an integrin antagonist for the treatment of cancer, as described in U.S. Serial No. 09/055,487, filed April 6, 1998, and WO 98/44797, published on October 15, 1998, which are incorporated herein by reference.

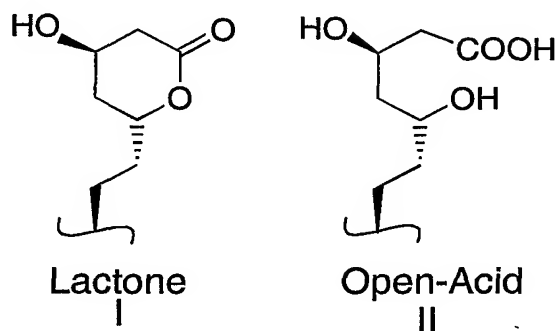
As used herein the term an integrin antagonist refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to an integrin(s) that is involved in the regulation of angiogenesis, or in the growth and invasiveness of tumor cells. In particular, the term refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to

the $\alpha v\beta 3$ integrin, which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha v\beta 5$ integrin, which antagonize, inhibit or counteract binding of a physiological ligand to both the $\alpha v\beta 3$ integrin and the $\alpha v\beta 5$ integrin, or which antagonize, inhibit or counteract the activity of the particular integrin(s) expressed on capillary endothelial cells. The term also refers to antagonists of the $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 5\beta 1$, $\alpha 6\beta 1$ and $\alpha 6\beta 4$ integrins. The term also refers to antagonists of any combination of $\alpha v\beta 3$ integrin, $\alpha v\beta 5$ integrin, $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 5\beta 1$, $\alpha 6\beta 1$ and $\alpha 6\beta 4$ integrins. The instant compounds may also be useful with other agents that inhibit angiogenesis and thereby inhibit the growth and invasiveness of tumor cells, including, but not limited to angiostatin and endostatin.

The instant compounds may also be useful in combination with an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) for the treatment of cancer. Compounds which have inhibitory activity for HMG-CoA reductase can be readily identified by using assays well-known in the art. For example, see the assays described or cited in U.S. Patent 4,231,938 at col. 6, and WO 84/02131 at pp. 30-33. The terms "HMG-CoA reductase inhibitor" and "inhibitor of HMG-CoA reductase" have the same meaning when used herein.

Examples of HMG-CoA reductase inhibitors that may be used include but are not limited to lovastatin (MEVACOR®; see US Patent No. 4,231,938; 4,294,926; 4,319,039), simvastatin (ZOCOR®; see US Patent No. 4,444,784; 4,820,850; 4,916,239), pravastatin (PRAVACHOL®; see US Patent Nos. 4,346,227; 4,537,859; 4,410,629; 5,030,447 and 5,180,589), fluvastatin (LESCOL®; see US Patent Nos. 5,354,772; 4,911,165; 4,929,437; 5,189,164; 5,118,853; 5,290,946; 5,356,896), atorvastatin (LIPITOR®; see US Patent Nos. 5,273,995; 4,681,893; 5,489,691; 5,342,952) and cerivastatin (also known as rivastatin and BAYCHOL®; see US Patent No. 5,177,080). The structural formulas of these and additional HMG-CoA reductase inhibitors that may be used in the instant methods are described at page 87 of M. Yalpani, "Cholesterol Lowering Drugs", *Chemistry & Industry*, pp. 85-89 (5 February 1996) and US Patent Nos. 4,782,084 and 4,885,314. The term HMG-CoA reductase inhibitor as used herein includes all pharmaceutically acceptable lactone and open-acid forms (i.e., where the lactone ring is opened to form the free acid) as well as salt and ester forms of compounds which have HMG-CoA reductase inhibitory activity, and therefor the use of such salts, esters, open-acid and lactone forms is included within the scope of this

invention. An illustration of the lactone portion and its corresponding open-acid form is shown below as structures I and II.



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- In HMG-CoA reductase inhibitors where an open-acid form can exist, salt and ester forms may preferably be formed from the open-acid, and all such forms are included within the meaning of the term "HMG-CoA reductase inhibitor" as used herein. Preferably, the HMG-CoA reductase inhibitor is selected from lovastatin and
- 10 simvastatin, and most preferably simvastatin. Herein, the term "pharmaceutically acceptable salts" with respect to the HMG-CoA reductase inhibitor shall mean non-toxic salts of the compounds employed in this invention which are generally prepared by reacting the free acid with a suitable organic or inorganic base, particularly those
- 15 formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc and tetramethylammonium, as well as those salts formed from amines such as ammonia, ethylenediamine, N-methylglucamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chlorprocaine, diethanolamine, procaine, N-benzylphenethylamine, 1-p-chlorobenzyl-2-pyrrolidine-1'-yl-methyl-benzimidazole, diethylamine, piperazine, and tris(hydroxymethyl) aminomethane.
- 20 Further examples of salt forms of HMG-CoA reductase inhibitors may include, but are not limited to, acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinatate, hydrabamine,
- 25 hydrobromide, hydrochloride, hydroxynapthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, oleate, oxalate, pamaote, palmitate, panthothenate, phosphate/

diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate.

Ester derivatives of the described HMG-CoA reductase inhibitor compounds may act as prodrugs which, when absorbed into the bloodstream of a warm-blooded animal, may cleave in such a manner as to release the drug form and permit the drug to afford improved therapeutic efficacy.

Similarly, the instant compounds may be useful in combination with agents that are effective in the treatment and prevention of NF-1, restenosis, polycystic kidney disease, infections of hepatitis delta and related viruses and fungal infections.

If formulated as a fixed dose, such combination products employ the combinations of this invention within the dosage range described above and the other pharmaceutically active agent(s) within its approved dosage range. Combinations of the instant invention may alternatively be used sequentially with known pharmaceutically acceptable agent(s) when a multiple combination formulation is inappropriate.

The instant compounds may also be useful in combination with prodrugs of antineoplastic agents. In particular, the instant compounds may be co-administered either concurrently or sequentially with a conjugate (termed a "PSA conjugate") which comprises an oligopeptide, that is selectively cleaved by enzymatically active prostate specific antigen (PSA), and an antineoplastic agent. Such co-administration will be particularly useful in the treatment of prostate cancer or other cancers which are characterized by the presence of enzymatically active PSA in the immediate surrounding cancer cells, which is secreted by the cancer cells.

Compounds which are PSA conjugates and are therefore useful in such a co-administration, and methods of synthesis thereof, can be found in the following patents, pending patent applications and publications which are herein incorporated by reference:

U.S. Patent No. 5,599,686, granted on February 4, 1997;

WO 96/00503 (January 11, 1996); USSN 08/404,833, filed on March 15, 1995;
USSN 08/468,161, filed on June 6, 1995;

U.S. Patent No. 5,866,679, granted on February 2, 1999;

WO 98/10651 (March 19, 1998); USSN 08/926,412, filed on September 9, 1997;

WO 98/18493 (May 7, 1998); USSN 08/950,805, filed on October 14, 1997;

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WO 99/02175 (January 21, 1999); USSN 09/112,656, filed on July 9, 1998; and

WO 99/28345 (June 10, 1999); USSN 09/193,365, filed on November 17, 1998.

10 Compounds which are described as prodrugs wherein the active therapeutic agent is released by the action of enzymatically active PSA and therefore may be useful in such a co-administration, and methods of synthesis thereof, can be found in the following patents, pending patent applications and publications, which are herein incorporated by reference: WO 98/52966 (November 26, 1998).

15 All patents, publications and pending patent applications identified are herein incorporated by reference.

 The compounds of the instant invention are also useful as a component in an assay to rapidly determine the presence and quantity of farnesyl-protein transferase (FPTase) in a composition. Thus the composition to be tested may be divided
20 and the two portions contacted with mixtures which comprise a known substrate of FPTase (for example a tetrapeptide having a cysteine at the amine terminus) and farnesyl pyrophosphate and, in one of the mixtures, a compound of the instant invention. After the assay mixtures are incubated for an sufficient period of time, well known in the art, to allow the FPTase to farnesylate the substrate, the chemical
25 content of the assay mixtures may be determined by well known immuno-logical, radiochemical or chromatographic techniques. Because the compounds of the instant invention are selective inhibitors of FPTase, absence or quantitative reduction of the amount of substrate in the assay mixture without the compound of the instant invention relative to the presence of the unchanged substrate in the assay containing
30 the instant compound is indicative of the presence of FPTase in the composition to be tested.

 It would be readily apparent to one of ordinary skill in the art that such an assay as described above would be useful in identifying tissue samples which contain farnesyl-protein transferase and quantitating the enzyme. Thus,

potent inhibitor compounds of the instant invention may be used in an active site titration assay to determine the quantity of enzyme in the sample. A series of samples composed of aliquots of a tissue extract containing an unknown amount of farnesyl-protein transferase, an excess amount of a known substrate of FPTase (for example a tetrapeptide having a cysteine at the amine terminus) and farnesyl pyrophosphate are incubated for an appropriate period of time in the presence of varying concentrations of a compound of the instant invention. The concentration of a sufficiently potent inhibitor (i.e., one that has a K_i substantially smaller than the concentration of enzyme in the assay vessel) required to inhibit the enzymatic activity of the sample by 50% is approximately equal to half of the concentration of the enzyme in that particular sample.

EXAMPLES

Examples provided are intended to assist in a further understanding of the invention. Particular materials employed, species and conditions are intended to be further illustrative of the invention and not limitative of the reasonable scope thereof.

EXAMPLE 1

Preparation of 5-[(17R)-10-cyano-14R-methyl-2,2-dioxido-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[15.3.1.1^{3,7}.1^{9,13}]tricoso-3(23),4,6,9(22),10,12-hexaen-14-yl]-1-methyl-1H-imidazol-1-ium trifluoroacetate and 5-[(17S)-10-cyano-14R-methyl-2,2-dioxido-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[15.3.1.1^{3,7}.1^{9,13}]tricoso-3(23),4,6,9(22),10,12-hexaen-14-yl]-1-methyl-1H-imidazol-1-ium trifluoroacetate

Step A: Preparation of 4-Bromo-3-fluorobenzoic acid

4-Bromo-3-fluorotoluene (40.0 g, 0.212 mol) was heated at 90°C in H₂O (200 mL)-pyridine (200 mL) with mechanical stirring under Ar. Potassium permanganate (KMnO₄) (67 g, 0.424 mol) was added portionwise over 3 hours. After 4 hours, an HPLC of a filtered sample indicated 50% conversion to the acid. An additional 30 g of KMnO₄ was added and heating continued overnight. HPLC indicated 81% conversion. Further KMnO₄ was added portionwise with reaction monitoring by HPLC until >95% conversion was obtained. The reaction mixture was

filtered through Celite, the filter pad washed with H₂O, aq NaOH and EtOH. The filtrate was concentrated to a small volume, then partitioned between 3N NaOH solution and diethyl ether. The aqueous basic layer was separated, cooled in an ice-H₂O bath and acidified slowly with 6N HCl solution to precipitate the white solid product. This was collected by suction filtration and dried at 40°C. in a vacuum oven overnight to give the title compound. mp 190 -192°C. ¹H NMR (CDCl₃) δ 7.83 (dd, 1H, J = 2, 9 Hz), 7.78 (dd, 1H, J = 2, 8 Hz), 7.67 - 7.71 (m, 1H).

Step B: Preparation of 4-bromo-3-fluorobenzyl alcohol

4-Bromo-3-fluorobenzoic acid (40.8 g, 0.187 mol) was dissolved in THF (250 ml) with magnetic stirring under Ar in an ice-H₂O bath. The cloudy solution was treated dropwise with borane-THF complex (1 M) (374 mL, 0.374 mol) over a 1 hour period maintaining the internal temperature at <10°C. The reaction mixture was left to warm to ambient temperature overnight, then cooled in an ice-H₂O bath and treated dropwise with H₂O (150 mL). The THF was removed on a rotary evaporator, and the residue partitioned between EtOAc and H₂O. The aqueous layer was extracted with EtOAc (3 x 100 mL), the organic layers combined, washed with brine, and dried (Na₂SO₄), filtered, and concentrated to give the title compound as an oil which solidified on standing. ¹H NMR (CDCl₃) δ 7.52 (t, 1H, J = 8 Hz), 7.16 (d, 1H, J = 9 Hz), 7.02 (d, 1H, J = 8 Hz), 4.67 (s, 2H), 1.47 (br s, 1H).

Step C: Preparation of 2-fluoro-4-hydroxymethylbenzonitrile

4-Bromo-3-fluorobenzyl alcohol (20 g, 0.097 mol) was dissolved in DMF (100 mL) then placed under high vacuum for 15 min. The solution was then purged with Ar for 15 min. While purging continued, zinc cyanide (8 g, 0.068 mol) and the catalyst, Pd[(PPh₃)₄], (5.63 g, 0.0049 mol) were added. The reaction mixture was heated at 95°C. under Ar for 18 h, then cooled to ambient temperature and added to H₂O. The mixture was extracted with EtOAc, then washed with 1M HCl, H₂O, brine, and dried (Na₂SO₄). Filtration and concentration to dryness gave the title compound as a white solid after chromatography (silica gel, hexane: EtOAc, 6.5:3.5). ¹H NMR (CDCl₃) δ 7.61 (t, 1H, J = 8 Hz), 7.23 - 7.29 (m, 2H), 4.80 (d, 2H, J = 6 Hz), 1.93 (t, 1H, J = 6Hz).

Step D: Preparation of 2-Fluoro-4-formylbenzonitrile

2-Fluoro-4-hydroxymethylbenzonitrile (10 g, 0.066 mol) and triethylamine (32.3 mL, 0.231 mol) were dissolved in CH₂Cl₂ (100 mL)- DMSO (20 mL) at <5°C with stirring and treated dropwise with a solution of pyridine•SO₃ complex (31.5 g, 0.198 mol) in DMSO (70 mL) maintaining the reaction mixture temperature at <10°C. The reaction mixture was stirred at 5°C for 1 hour after the addition, then at 20°C for 1 hour, then partitioned between CH₂Cl₂ and H₂O. The organic layer was separated, washed well with H₂O, brine, and dried (Na₂SO₄). Filtration and concentration gave the title compound after purification by chromatography (silica gel, hexane: EtOAc, 3:1). ¹H NMR (CDCl₃) δ 10.06 (d, 1H, J = 2 Hz), 7.86 (dd, 1H, J = 5,8 Hz), 7.798 (dd, 1H, J = 1, 8 Hz), 7.728 (dd, 1H, J = 1, 8 Hz).

Step E: Preparation of 2-Fluoro-4-[hydroxy-(3-methyl-3H-imidazol-4-yl)-methyl]-benzonitrile

1-Methylimidazole (15.88 mL, 0.199 mol), dissolved in anhydrous THF (500 mL) in flame-dried glassware under Ar, was cooled to -78°C and treated with n-butyl lithium (1.6M in hexane)(124 mL, 0.199 mol) via syringe. After stirring for 1 hour chlorotriethylsilane (33.4 mL, 0.199 mol) was added and the reaction mixture was left to warm to ambient temperature overnight. The THF was removed *in vacuo* with gentle warming, and the residue was redissolved in dry THF (500 mL), cooled to -78°C, and treated with sec-butyl lithium (1.3M in cyclohexane) (153 mL, 0.199 mol) dropwise. After 1 hour this solution was cannulated into a solution of 2-fluoro-4-formylbenzonitrile (27 g, 0.181 mol) in THF (200 mL). After 15 minutes the cooling bath was removed, the mixture was stirred for 2 hr at ambient temperature, then was quenched with saturated NH₄Cl solution. After 15 minutes 10% HCl was added to pH = 3. After 0.5 hour the THF was removed *in vacuo*, the mixture was made basic with solid Na₂CO₃ and extracted with EtOAc (3 x 200 mL). The organics were combined, washed with 10% HCl (3x), the aqueous acidic layers combined, made basic with solid Na₂CO₃, extracted with EtOAc (3x), the organics combined, washed with brine, and dried (MgSO₄). Filtration and concentration to dryness gave the title compound.

Step F: Preparation of 2-Fluoro-4-(3-methyl-3H-imidazole-4-carbonyl)-benzonitrile

2-Fluoro-4-[hydroxy-(3-methyl-3*H*-imidazol-4-yl)-methyl]-benzonitrile (0.655 g, 2.83 mmol) and MnO₂ (1.23 g, 14.2 mmol) were stirred in CH₂Cl₂ (50 mL) and CH₃CN (5 mL) for 3 hours. The solution was filtered and concentrated to yield the title compound.

5

Step G: Preparation of *N*-[(4-cyano-3-fluoro-phenyl)-(3-methyl-3*H*-imidazol-4-yl)-methylene]-2-methylpropanesulfinamide

2-Fluoro-4-(3-methyl-3*H*-imidazole-4-carbonyl)-benzonitrile (2.56 g, 11.2 mmol), titanium(IV) ethoxide (7.02 mL, 33.5 mmol) and commercially available (R)-(+)-2-methyl-2-propanesulfinamide (1.35 g, 11.17 mmol) were dissolved in anhydrous THF (100 mL) and heated at 75°C for 7 days. The solution was cooled, diluted with brine (100 mL), filtered through a celite pad and washed generously with EtOAc and H₂O. The filtrate was separated, dried (MgSO₄), and purified using SiO₂ chromatography (0-3% MeOH/CH₂Cl₂) to give the title compound.

15

Step H: Preparation of *N*-[1-(4-cyano-3-fluoro-phenyl)-1-(3-methyl-3*H*-imidazol-4-yl)-ethyl]-2-methylpropanesulfinamide

N-[(4-Cyano-3-fluoro-phenyl)-(3-methyl-3*H*-imidazol-4-yl)-methylene]-2-methylpropanesulfinamide (1.50 g, 4.51 mmol) was dissolved in anhydrous THF (30 mL) at 0°C and treated with a 3.0M solution of MeMgBr (4.50 mL, 13.5 mmol) in Et₂O. After 15 minutes the reaction was quenched with aq. NH₄Cl solution, diluted with saturated NaHCO₃ solution and extracted with CH₂Cl₂ (3X). The combined organic layers were dried (MgSO₄), filtered, concentrated to give the title compound.

25

Step I: Preparation of 4-[(1*S*)-amino-1-(3-methyl-3*H*-imidazol-4-yl)-ethyl]-2-fluoro-benzonitrile

A cold methanolic HCl solution (50 mL) was added to *N*-[1-(4-cyano-3-fluoro-phenyl)-1-(3-methyl-3*H*-imidazol-4-yl)-ethyl]-2-methylpropanesulfinamide (0.880 g, 2.51 mmol) dissolved in MeOH (50 mL) and stirred for 1 hour at room temperature. After concentration, the crude product was chromatographed using a Chiralcel OD column eluting with 60-100% EtOH in hexane (0.1% DEA) to give the title compound as the major enantiomer.

Using the procedure described above, but substituting (S)-(-)-2-methyl-2-propanesulfinamide for (R)-(+)-2-methyl-2-propanesulfinamide in Step

G, 4-[(1R)-amino-1-(3-methyl-3*H*-imidazol-4-yl)-ethyl]-2-fluoro-benzonitrile was obtained as the major enantiomer.

5 Step J: Preparation of 1-(3-methoxy-benzenesulfonyl)-piperidine-3-carboxylic acid ethyl ester

A solution of ethyl pipercolate (1.02 g, 6.46 mmol) and Et₃N (1.98 mL, 14.2 mmol) in CH₂Cl₂ (13 mL) is treated with 3-methoxybenzenesulfonyl chloride (1.33 g, 6.46 mmol) dropwise with stirring under N₂ at 0°C. After 3 hours the reaction was quenched with H₂O, extracted with CH₂Cl₂ (2x), the organics
10 combined, washed with brine, dried (Na₂SO₄), filtered and concentrated to give the title compound as a yellow oil.

15 Step K: Preparation of 1-(3-methoxy-benzenesulfonyl)-piperidine-3-carboxylic acid

To a solution of 1-(3-methoxy-benzenesulfonyl)-piperidine-3R-carboxylic acid ethyl ester (2.02 g, 6.16 mmol) in absolute ethanol (20 mL) at ambient temperature was added 50% w/w NaOH (0.26 mL, 6.47 mmol) and H₂O (1.25 mL). The reaction was stirred overnight, then treated with 12N HCl (0.53 mL) to a pH = 4. The ethanol was removed *in vacuo* and the residue partitioned between
20 EtOAc and H₂O. The aqueous layer was washed with EtOAc (2x), the organics combined, washed with brine, dried (Na₂SO₄), filtered, and concentrated *in vacuo*. Under the basic hydrolysis conditions partial racemization resulted in a 3:1 mixture of R:S enantiomers, respectively. LC/MS (M+1) 300.

25 Step L: Preparation of 3-*N*-[1-(4-cyano-3-fluorophenyl)-1-(1-methyl-1*H*-imidazol-5-yl)ethyl]-1-[(3-methoxyphenyl)sulfonyl] piperidine-3-carboxamide

4-[(1R)-amino-1-(3-methyl-3*H*-imidazol-4-yl)-ethyl]-2-fluoro-benzonitrile (Step I) (0.192 g, 0.78 mmol), 1-(3-methoxy-benzenesulfonyl)-
30 piperidine-3-carboxylic acid, (0.235 g, 0.78 mmol), EDC (0.284 g, 1.5 mmol), HOBt (0.230 g, 1.5 mmol) and N-methylmorpholine (0.172 mL, 1.5 mmol) were stirred in DMF (4 mL) at ambient temperature overnight under N₂. The reaction mixture was concentrated *in vacuo*, then partitioned between EtOAc and H₂O, the aqueous layer washed with EtOAc (2x), the organics combined, washed with
35 brine, and dried (Na₂SO₄), filtered, and concentrated *in vacuo* to provide the title

compound after normal phase SiO₂ chromatography on an ISCO combiflash eluting with 1-5% CH₃OH:CH₂Cl₂ (0.1% NH₄OH). LC/MS (M+1) 525.

5 Step M: Preparation of 3-*N*-[1-(4-cyano-3-fluorophenyl)-1-(1-methyl-1*H*-imidazol-5-yl)ethyl]-1-[(3-hydroxyphenyl)sulfonyl] piperidine-3-carboxamide

10 3-*N*-[1-(4-cyano-3-fluorophenyl)-1-(1-methyl-1*H*-imidazol-5-yl)ethyl]-1-[(3-methoxyphenyl)sulfonyl] piperidine-3-carboxamide (0.265 g, 0.42 mmol) was dissolved in CH₂Cl₂ (13 mL) at 0°C. and treated with 1M BBr₃ in

15 CH₂Cl₂ (8.42 mL, 8.42 mmol). After 1 hour aqueous saturated NaHCO₃ solution was added and, the layers were separated. The aqueous layer extracted with CH₂Cl₂ (3x), and the organics combined, washed with brine, and dried (Na₂SO₄). Filtration and concentration to dryness gave crude product which was combined with a gummy residue which was insoluble in both phases and chromatographed on RPLC PrepPak eluting with 0.1%TFA-H₂O:0.1%TFA-CH₃CN, 95:5 to 5:95 to give the title compound as the TFA salt after lyophilization. LC/MS (M+1) 511.

20 Step N: Preparation of 5-[(17*R*)-10-cyano-14*R*-methyl-2,2-dioxido-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[15.3.1.1^{3,7}.1^{9,13}]tricoso-3(23),4,6,9(22),10,12-hexaen-14-yl]-1-methyl-1*H*-imidazol-1-ium trifluoroacetate

25 To a solution of 3-*N*-[1-(4-cyano-3-fluorophenyl)-1-(1-methyl-1*H*-imidazol-5-yl)ethyl]-1-[(3-hydroxyphenyl)sulfonyl] piperidine-3-carboxamide (0.243 g, 0.39 mmol) in DMF (10 mL) was added cesium carbonate (0.569 g, 1.75 mmol). The mixture was stirred at 60°C for 17 hours, taken up in water, concentrated to a small volume, and purified on a Waters Delta PrepPak HPLC column using a 0.1% trifluoroacetic acid/water: 0.1% trifluoroacetic acid/acetonitrile, 95:5 to 5:95 gradient over 1 hour. The pure fractions were pooled, concentrated to remove the acetonitrile and lyophilized overnight to yield 5-[(17*R*)-10-cyano-14*R*-methyl-2,2-

30 dioxido-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[15.3.1.1^{3,7}.1^{9,13}]tricoso-3(23),4,6,9(22),10,12-hexaen-14-yl]-1-methyl-1*H*-imidazol-1-ium trifluoroacetate as the major diastereomer, FAB mass spectrum m/e 491 (M+1), and 5-[(17*S*)-10-cyano-14*R*-

methyl-2,2-dioxido-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[15.3.1.13.7.19,13]tricoso-3(23),4,6, 9(22),10,12-hexaen-14-yl]-1-methyl-1*H*-imidazol-1-ium trifluoroacetate as the minor diastereomer, FAB mass spectrum *m/e* 491 (*M*+1).

5 These compounds, as free bases, are also named as (14*R*,17*R* or *S*)-14-methyl-14-(1-methyl-1*H*-imidazol-5-yl)-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[15.3.1.1^{3,7}.1^{9,13}] tricoso-3(23),4,6,9(22),10,12-hexaene-10-carbonitrile 2,2-dioxide.

10 EXAMPLE 2

Preparation of 5-[(18S)-10-cyano-14-methyl-2,2-dioxido-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[16.3.1.1^{3,7}.1^{9,13}]tetracos-3(24),4,6,9(23),10,12-hexaen-14-yl]-1-methyl-1H-imidazol-1-ium trifluoroacetate

15 Using the procedures described in Example 1, but substituting 1-(3-methoxy-benzenesulfonyl)-piperidine-3-acetic acid for 1-(3-methoxy-benzenesulfonyl)-piperidine-3-carboxylic acid in Step L, the title compound was prepared. LC/MS M+1 506.

This compound, as free bases, is also named as (18*S*)-14-methyl-14-(1-methyl-1*H*-imidazol-5-yl)-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[16.3.1.1^{3,7}.1^{9,13}]tetracos-3(24),4,6,9(23),10,12-hexaene-10-carbonitrile 2,2-dioxide trifluoroacetate salt.

EXAMPLE 3

25 Preparation of 5-[(19*S*)-10-cyano-14-methyl-2,2-dioxido-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[17.3.1.1³.7.19,¹³]pentacosa-3(25),4,6,9(24),10,12-hexaen-14-yl]-1-methyl-1*H*-imidazol-1-ium trifluoroacetate and 5-[(19*R*)-10-cyano-14-methyl-2,2-dioxido-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[17.3.1.1³.7.19,¹³]pentacosa-3(25),4,6,9(24),10,12-hexaen-14-yl]-1-methyl-1*H*-imidazol-1-ium trifluoroacetate
30

Using the procedures described in Example 1, but substituting 1-(3-methoxy-benzenesulfonyl)-piperidine-3-propionic acid for 1-(3-methoxy-benzenesulfonyl)-piperidine-3-carboxylic acid in Step L, the title compounds were prepared. LC/MS M+1 520.

35 These compounds, as free bases, are also named as (19*S*)-14-

methyl-14-(1-methyl-1H-imidazol-5-yl)-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo
[17.3.1.1^{3,7}.1^{9,13}]pentacosa-3(25),4,6,9(24),10,12-hexaene-10-carbonitrile 2,2-dioxide
and (19*R*)-14-methyl-14-(1-methyl-1H-imidazol-5-yl)-16-oxo-8-oxa-2-thia-1,15-
diazatetracyclo[17.3.1.1^{3,7}.1^{9,13}]pentacosa-3(25),4,6,9(24),10,12-hexaene-10-
5 carbonitrile 2,2-dioxide.

EXAMPLE 4

Preparation of (6*S*)-11*S*-amino-11-(1-methyl-1H-imidazol-5-yl)-17-oxa-2-thia-3,7-
10 diazatetracyclo[16.3.1.1^{3,6}.1^{12,16}]tetracosa-1(22),12(23),13,15,18,20-hexaene-15-
carbonitrile 2,2-dioxide

Step A: Preparation of Benzyl 3-bromophenyl ether

To a stirred solution of 3-bromophenol (9.50 g, 54.9 mmol) in
15 degassed DMF (150 mL) at 0°C, under argon, was added Cs₂CO₃ (35.8 g, 109
mmol). The resulting mixture was stirred for 1 hour, then benzyl bromide (10.3 g,
60.2 mmol) was added and stirring was continued for 2 hours at 0°C. The solvent
was removed under reduced pressure, and the residue was partitioned between
20% aqueous NaOH (250 mL) and CHCl₃ (500 mL). The organic layer was dried
20 over MgSO₄, filtered, and concentrated *in vacuo*. The crude product was purified
by flash column chromatography on silica, eluting first with two column volumes of
hexane, then with hexane - 2% EtOAc to yield the desired product as a white solid.

Step B: Preparation of Magnesium 3-benzyloxyphenylsulfinate bromide

To a stirred suspension of Rieke Mg (0.5 g, 21 mmol) in refluxing
25 dry THF (45 mL), under argon, was added benzyl 3-bromophenyl ether, as described
above in Step A, (5.0 g, 19 mmol) in dry THF (50 mL) dropwise, at a rate that main-
tained reflux with the heat source removed. The resulting mixture was heated to
reflux for 30 min, then cooled to -78°C and SO₂ was bubbled into the solution
30 slowly for 1 hr. The reaction mixture was allowed to warm slowly to ambient
temperature and stirred for 18 hours. Argon was bubbled into the mixture for 1 hour,
then the solution was concentrated *in vacuo* to give the desired product as a solid.

Step C: Preparation of 3-Benzyloxyphenylsulfonyl chloride

Magnesium 3-benzyloxyphenylsulfinate bromide, as described above in Step B, (4.5 g, 14 mmol) was dissolved in dry CH₂Cl₂ (175 mL) and cooled to 0°C under argon. Sulfuryl chloride (19 mL of a 1.0 M solution in CH₂Cl₂, 19 mmol) was added dropwise, and the mixture was stirred for 18 hrs at ambient temperature, during which time a precipitate formed. The precipitate was removed by filtration and the filtrate was concentrated under reduced pressure to give the product as a yellow oil.

10 Step D: Preparation of (R)-1-(3-Benzyloxyphenylsulfonyl)-3-(*tert*-butoxycarbonylamino)pyrrolidine

To a stirred solution of 3-benzyloxyphenylsulfonyl chloride, as described above in Step C, (1.8 g, 7.2 mmol) in CH₂Cl₂ (50 mL) was added *N,N*-diisopropylethylamine (1.8 mL, 10.3 mmol) followed by (R)-3-(*tert*-butoxycarbonylamino)pyrrolidine (1.77 g, 9.5 mmol) and the reaction mixture was stirred at ambient temperature for 4 hours. The mixture was washed with 10% aqueous citric acid (75 mL), then water (50 mL), then saturated aqueous NaHCO₃ (50 mL). The organic layer was dried over MgSO₄, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica, eluting with hexane - 20% EtOAc to yield the desired product as a white solid.

20 Step E: Preparation of (R)-3-(*tert*-Butoxycarbonylamino)-1-(3-hydroxyphenylsulfonyl)pyrrolidine

To a solution of (R)-1-(3-benzyloxyphenylsulfonyl)-3-(*tert*-butoxycarbonylamino)pyrrolidine, as described above in Step D, (3.1 g, 7.17 mmol) in EtOH (150 mL) was added 20% Pd(OH)₂ on carbon (900 mg) and the reaction mixture was stirred under an atmosphere of hydrogen (ca. 1 atm) at ambient temperature for 18 hours. The mixture was filtered through a pad of celite, washing with EtOH, and the filtrate was concentrated *in vacuo* to give the titled product as a white solid.

30 Step F: Preparation of (R)-3-Amino-1-(3-hydroxyphenyl-sulfonyl)pyrrolidine Hydrochloride

A solution of (R)-3-(*tert*-butoxycarbonylamino)-1-(3-hydroxyphenyl-sulfonyl)pyrrolidine, as described above in Step E, (2.4 g, 7.0 mmol) in EtOAc (100 mL) at 0°C was saturated with HCl (g). After 15 minutes, the mixture was concentrated *in vacuo* to yield the amine hydrochloride as a white solid.

Using the methods outlined above, but substituting (*S*)-3-(*tert*-butoxycarbonylamino)pyrrolidine for (*R*)-3-(*tert*-butoxycarbonyl-amino)pyrrolidine in Step D, (*S*)-3-Amino-1-(3-hydroxyphenylsulfonyl)pyrrolidine hydrochloride was prepared.

5

Step G: Preparation of N-[4-{[*tert*-butyl(dimethyl)silyl]oxy}-1-(4-cyano-3-fluorophenyl)-1-(1-methyl-1H-imidazol-5-yl)butyl]-2-methylpropane-2-sulfinamide

To a flame-dried flask containing magnesium (0.317 g, 12.03 mmol) under N₂ was added dry THF (3 mL), a pinch of iodine, and Rieke magnesium (1 mL of a 1M THF solution). 3-*tert*-Butyl(dimethyl)silyloxypropylbromide (3.048 g, 12.03 mmol) in THF (5 mL) was added in a small portion to the magnesium suspension with gentle warming to initiate the reaction. Once the reaction had started, the rest of the bromide solution was added dropwise over 15 minutes. After stirring for 15 minutes, this solution was added to a solution of *N*-[(4-cyano-3-fluoro-phenyl)-(3-methyl-3*H*-imidazol-4-yl)-methylene]-2-methylpropanesulfinamide (Example 1, Step G) (1.00 g, 3.01 mmol) in dry THF (12 mL) with stirring in an ice-H₂O bath under N₂. Grignard solution was added until HPLC indicated loss of starting imine. The reaction mixture was quenched with H₂O and partitioned between aqueous saturated NaHCO₃ solution and CH₂Cl₂. The organic layer was separated, the aqueous layer washed with CH₂Cl₂ (2x), the organics combined, washed with brine, dried (MgSO₄), filtered and concentrated to give the title compound after purification on an ISCO combiflash column eluting with 1-3% CH₃OH/CH₂Cl₂ (NH₄OH).

25 Step H: Preparation of N-[1-(4-cyano-3-fluorophenyl)-4-hydroxy-1(*S* and *R*)-(1-methyl-1H-imidazol-5-yl)butyl]-2-methylpropane-2-sulfinamide
N-[4-{[*tert*-Butyl(dimethyl)silyl]oxy}-1-(4-cyano-3-fluorophenyl)-1-(1-methyl-1H-imidazol-5-yl)butyl]-2-methylpropane-2-sulfinamide (0.111 g, 0.219 mmol) was dissolved in CH₃CN (80 mL): H₂O (20 mL) at ambient temperature and treated with CF₃CO₂H (1 mL) with stirring. After 0.5 hour the mixture was neutralized with aqueous saturated NaHCO₃ solution and concentrated to remove the CH₃CN. The crude product was chromatographed on a Chiralcel AD HPLC column eluting with 90/10 hexane/ethanol to give both enantiomers: N-[1-(4-cyano-3-fluorophenyl)-4-hydroxy-1(*S*)-(1-methyl-1H-imidazol-5-yl)butyl]-2-methylpropane-

2-sulfinamide MS (M+1) 393; and N-[1-(4-cyano-3-fluorophenyl)-4-hydroxy-1(R)-(1-methyl-1H-imidazol-5-yl)butyl]-2-methylpropane-2-sulfinamide MS (M+1) 393.

Step I: Preparation of N-[1-(4-cyano-3-fluorophenyl)-1-(1-methyl-1H-imidazol-5-yl)-4-oxobutyl]-2-methylpropane-2-sulfinamide

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- Pyridine-SO₃ complex (0.174 g, 1.10 mmol) in DMSO (2 mL) was added to a solution of N-[1-(4-cyano-3-fluorophenyl)-4-hydroxy-1(S)-(1-methyl-1H-imidazol-5-yl)butyl]-2-methylpropane-2-sulfinamide (0.108 g, 0.275 mmol) and Et₃N (0.222 g, 2.2 mmol) in CH₂Cl₂ (5 mL) with cooling in an ice-H₂O bath. The reaction mixture was allowed to warm to ambient temperature, stirred overnight, then partitioned between aqueous saturated NaHCO₃ solution and CH₂Cl₂. The aqueous layer was washed with CH₂Cl₂ (3x), organics combined, washed with brine, dried (MgSO₄), filtered and concentrated to dryness to give the title compound after purification on an ISCO combiflash eluting with 1-5% CH₃OH/CH₂Cl₂.
LC/MS = 391.

Step J: Preparation of N-[1-(4-cyano-3-fluorophenyl)-4-((3S)-1-[(3-hydroxyphenyl)sulfonyl]pyrrolidin-3-yl)amino)-1-(1-methyl-1H-imidazol-5-yl)butyl]-2-methylpropane-2-sulfinamide

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- N-[1-(4-Cyano-3-fluorophenyl)-1-(1-methyl-1H-imidazol-5-yl)-4-oxobutyl]-2-methylpropane-2-sulfinamide (0.028 g, 0.0717 mmol) and (S)-3-Amino-1-(3-hydroxyphenylsulfonyl) pyrrolidine hydrochloride (0.021 g, 0.0753 mmol) were dissolved in CH₃OH (5 mL) with stirring at ambient temperature, the pH of the solution adjusted to 5 with acetic acid, and the solution stirred for 0.5 hour. Sodium cyanoborohydride (0.0068 g, 0.1076 mmol) was added, and the mixture stirred for 16 hours. The mixture was concentrated *in vacuo* and partitioned between aqueous saturated NaHCO₃ solution and CH₂Cl₂. The aqueous layer was washed with CH₂Cl₂ (3x), organics combined, washed with brine, dried (MgSO₄), filtered and concentrated to dryness to give the title compound.

Step K: Preparation of N-[(6S)-15-cyano-11-(1-methyl-1H-imidazol-5-yl)-2,2-dioxido-17-oxa-2-thia-3,7-diazatetracyclo [16.3.1.13,6.112,16] tetracos-1(22),12(23),13,15,18,20-hexaen-11-yl]-2-methylpropane-2-sulfinamide

A solution of N-[1-(4-cyano-3-fluorophenyl)-4-((3S)-1-[(3-hydroxyphenyl)sulfonyl]pyrrolidin-3-yl)amino)-1-(1-methyl-1H-imidazol-5-yl)butyl]-2-methylpropane-2-sulfinamide (0.030 g, 0.0486 mmol) in DMF (5 mL) was treated with Cs₂CO₃ (0.0475 g, 0.1458 mmol) and heated overnight at 80°C.

- 5 The reaction mixture was concentrated *in vacuo*, partitioned between aqueous saturated NaHCO₃ solution and CH₂Cl₂. The aqueous layer was washed with CH₂Cl₂ (2x), organics combined, washed with brine, dried (MgSO₄), filtered and concentrated to dryness to give the title compound.

- 10 Step L: Preparation of (6S)-11-amino-11-(1-methyl-1H-imidazol-5-yl)-17-oxa-2-thia-3,7-diazatetracyclo [16.3.1.1^{3,6}.1^{12,16}]tetracos-1(22),12(23),13,15,18,20-hexaene-15-carbonitrile 2,2-dioxide
N-[(6S)-15-cyano-11-(1-methyl-1H-imidazol-5-yl)-2,2-dioxido-17-oxa-2-thia-3,7-diazatetracyclo [16.3.1.1^{3,6}.1^{12,16}]tetracos-1(22),12(23),13,15,18,20-hexaen-11-yl]-2-methylpropane-2-sulfinamide (0.0486 mmol) was dissolved in CH₃OH (5 mL) – 4M HCl in dioxane (4 mL), stirred for 15 min at ambient temperature, concentrated *in vacuo*, then chromatographed on RPLC PrepPak eluting with 95:5 to 5:95 H₂O:CH₃CN with 0.1% TFA over 60 minutes. Like fractions were combined, concentrated, and lyophilized to give the title compound.

- 20 MS (M+1) = 493.

Anal. calcd for C₂₅H₂₈N₆O₃S · 1.00 H₂O • 3.2 CF₃CO₂H:

C, 43.08; H, 3.82; N, 9.60;

Found: C, 42.76; H, 4.07; N, 9.65.

- 25 Using (R)-3-Amino-1-(3-hydroxyphenylsulfonyl) pyrrolidine hydrochloride in place of the S-enantiomer in Step J, (6R)-11R-amino-11-(1-methyl-1H-imidazol-5-yl)-17-oxa-2-thia-3,7-diazatetracyclo [16.3.1.1^{3,6}.1^{12,16}]tetracos-1(22),12(23),13,15,18,20-hexaene-15-carbonitrile 2,2-dioxide was prepared in a similar manner. MS (M+1) 493.

30

EXAMPLE 5

In vitro inhibition of ras farnesyl transferase

Transferase Assays. Isoprenyl-protein transferase activity assays are carried out at 30°C unless noted otherwise. A typical reaction contains (in a final volume of 50 µL): [³H]farnesyl diphosphate, Ras protein, 50 mM HEPES, pH 7.5, 5 mM MgCl₂, 5 mM dithiothreitol, 10 µM ZnCl₂, 0.1% polyethyleneglycol (PEG) (15,000-20,000 mw) and isoprenyl-protein transferase. The FPTase employed in the assay is prepared by recombinant expression as described in Omer, C.A., Kral, A.M., Diehl, R.E., Prendergast, G.C., Powers, S., Allen, C.M., Gibbs, J.B. and Kohl, N.E. (1993) *Biochemistry* 32:5167-5176. After thermally pre-equilibrating the assay mixture in the absence of enzyme, reactions are initiated by the addition of isoprenyl-protein transferase and stopped at timed intervals (typically 15 min) by the addition of 1 M HCl in ethanol (1 mL). The quenched reactions are allowed to stand for 15 minutes (to complete the precipitation process). After adding 2 mL of 100% ethanol, the reactions are vacuum-filtered through Whatman GF/C filters. Filters are washed four times with 2 mL aliquots of 100% ethanol, mixed with scintillation fluid (10 mL) and then counted in a Beckman LS3801 scintillation counter.

For inhibition studies, assays are run as described above, except inhibitors are prepared as concentrated solutions in 100% dimethyl sulfoxide and then diluted 20-fold into the enzyme assay mixture. Substrate concentrations for inhibitor IC₅₀ determinations are as follows: FTase, 650 nM Ras-CVLS (SEQ.ID.NO.: 2), 100 nM farnesyl diphosphate.

The compounds of the instant invention are tested for inhibitory activity against human FPTase by the assay described above.

The compounds of the instant case were tested by the assay above and were found to have an IC₅₀ of <1µM.

EXAMPLE 6

Modified *In vitro* GGTase inhibition assay

The modified geranylgeranyl-protein transferase inhibition assay is carried out at room temperature. A typical reaction contains (in a final volume of 50 µL): [³H]geranylgeranyl diphosphate, biotinylated Ras peptide, 50 mM HEPES, pH 7.5, a modulating anion (for example 10 mM glycerophosphate or 5mM ATP), 5 mM MgCl₂, 10 µM ZnCl₂, 0.1% PEG (15,000-20,000 mw), 2 mM dithiothreitol, and geranylgeranyl-protein transferase type I (GGTase). The GGTase-type I enzyme

employed in the assay is prepared as described in U.S. Patent No. 5,470,832, incorporated by reference. The Ras peptide is derived from the K4B-Ras protein and has the following sequence: biotinyl-GKKKKKKSKTKCVIM (single amino acid code) (SEQ.ID.NO.: 3). Reactions are initiated by the addition of GGTase and stopped at timed intervals (typically 15 min) by the addition of 200 μ L of a 3 mg/mL suspension of streptavidin SPA beads (Scintillation Proximity Assay beads, Amersham) in 0.2 M sodium phosphate, pH 4, containing 50 mM EDTA, and 0.5% BSA. The quenched reactions are allowed to stand for 2 hours before analysis on a Packard TopCount scintillation counter.

For inhibition studies, assays are run as described above, except inhibitors are prepared as concentrated solutions in 100% dimethyl sulfoxide and then diluted 25 fold into the enzyme assay mixture. IC₅₀ values are determined with Ras peptide near K_M concentrations. Enzyme and substrate concentrations for inhibitor IC₅₀ determinations are as follows: 75 pM GGTase-I, 1.6 μ M Ras peptide, 100 nM geranylgeranyl diphosphate.

The compounds of the instant invention are tested for inhibitory activity against human GGTase-type I by the assay described above.

EXAMPLE 7

Cell-based *in vitro* ras farnesylation assay

The cell line used in this assay is a v-ras line derived from either Rat1 or NIH3T3 cells, which expressed viral Ha-ras p21. The assay is performed essentially as described in DeClue, J.E. et al., Cancer Research 51:712-717, (1991). Cells in 10 cm dishes at 50-75% confluency are treated with the test compound (final concentration of solvent, methanol or dimethyl sulfoxide, is 0.1%). After 4 hours at 37°C, the cells are labeled in 3 ml methionine-free DMEM supplemented with 10% regular DMEM, 2% fetal bovine serum and 400 μ Ci [³⁵S]methionine (1000 Ci/mmol). After an additional 20 hours, the cells are lysed in 1 ml lysis buffer (1% NP40/20 mM HEPES, pH 7.5/5 mM MgCl₂/1mM DTT/10 mg/ml aprotinin/2 mg/ml leupeptin/2 mg/ml antipain/0.5 mM PMSF) and the lysates cleared by centrifugation at 100,000 x g for 45 minutes. Aliquots of lysates containing equal numbers of acid-precipitable counts are brought to 1 ml with IP buffer (lysis buffer lacking DTT) and immuno-precipitated with the ras-specific monoclonal antibody Y13-259 (Furth, M.E. et al., J. Virol. 43:294-304, (1982)). Following a 2 hour antibody incubation at

4°C, 200 µl of a 25% suspension of protein A-Sepharose coated with rabbit anti rat IgG is added for 45 minutes. The immuno-precipitates are washed four times with IP buffer (20 mM HEPES, pH 7.5/1 mM EDTA/1% Triton X-100.0.5% deoxycholate/0.1%/SDS/0.1 M NaCl) boiled in SDS-PAGE sample buffer and loaded on 13% acrylamide gels. When the dye front reached the bottom, the gel is fixed, soaked in Enlightening, dried and autoradiographed. The intensities of the bands corresponding to farnesylated and nonfarnesylated ras proteins are compared to determine the percent inhibition of farnesyl transfer to protein.

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EXAMPLE 8

Cell-based *in vitro* growth inhibition assay

To determine the biological consequences of FPTase inhibition, the effect of the compounds of the instant invention on the anchorage-independent growth of Rat1 cells transformed with either a v-ras, v-raf, or v-mos oncogene is tested. Cells transformed by v-Raf and v-Mos maybe included in the analysis to evaluate the specificity of instant compounds for Ras-induced cell transformation.

Rat 1 cells transformed with either v-ras, v-raf, or v-mos are seeded at a density of 1×10^4 cells per plate (35 mm in diameter) in a 0.3% top agarose layer in medium A (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum) over a bottom agarose layer (0.6%). Both layers contain 0.1% methanol or an appropriate concentration of the instant compound (dissolved in methanol at 1000 times the final concentration used in the assay). The cells are fed twice weekly with 0.5 ml of medium A containing 0.1% methanol or the concentration of the instant compound. Photomicrographs are taken 16 days after the cultures are seeded and comparisons are made.

EXAMPLE 9

Construction of SEAP reporter plasmid pDSE100

The SEAP reporter plasmid, pDSE100 was constructed by ligating a restriction fragment containing the SEAP coding sequence into the plasmid pCMV-RE-AKI. The SEAP gene is derived from the plasmid pSEAP2-Basic (Clontech, Palo Alto, CA). The plasmid pCMV-RE-AKI was constructed by Deborah Jones (Merck)

and contains 5 sequential copies of the 'dyad symmetry response element' cloned upstream of a 'CAT-TATA' sequence derived from the cytomegalovirus immediate early promoter. The plasmid also contains a bovine growth hormone poly-A sequence.

5 The plasmid, pDSE100 was constructed as follows. A restriction fragment encoding the SEAP coding sequence was cut out of the plasmid pSEAP2-Basic using the restriction enzymes EcoR1 and HpaI. The ends of the linear DNA fragments were filled in with the Klenow fragment of *E. coli* DNA Polymerase I. The 'blunt ended' DNA containing the SEAP gene was isolated by electrophoresing
10 the digest in an agarose gel and cutting out the 1694 base pair fragment. The vector plasmid pCMV-RE-AKI was linearized with the restriction enzyme Bgl-II and the ends filled in with Klenow DNA Polymerase I. The SEAP DNA fragment was blunt end ligated into the pCMV-RE-AKI vector and the ligation products were transformed into DH5-alpha *E. coli* cells (Gibco-BRL). Transformants were screened
15 for the proper insert and then mapped for restriction fragment orientation. Properly oriented recombinant constructs were sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid contains the SEAP coding sequence downstream of the DSE and CAT-TATA promoter elements and upstream of the BGH poly-A sequence.

20

Alternative Construction of SEAP reporter plasmid, pDSE101

The SEAP reporter plasmid, pDSE101 is also constructed by ligating a restriction fragment containing the SEAP coding sequence into the plasmid pCMV-RE-AKI. The SEAP gene is derived from plasmid pGEM7zf(-)/SEAP.

25 The plasmid pDSE101 was constructed as follows: A restriction fragment containing part of the SEAP gene coding sequence was cut out of the plasmid pGEM7zf(-)/SEAP using the restriction enzymes Apa I and KpnI. The ends of the linear DNA fragments were chewed back with the Klenow fragment of *E. coli* DNA Polymerase I. The "blunt ended" DNA containing the truncated SEAP
30 gene was isolated by electrophoresing the digest in an agarose gel and cutting out the 1910 base pair fragment. This 1910 base pair fragment was ligated into the plasmid pCMV-RE-AKI which had been cut with Bgl-II and filled in with *E. coli* Klenow fragment DNA polymerase. Recombinant plasmids were screened for insert orientation and sequenced through the ligated junctions. The plasmid pCMV-RE-AKI is
35 derived from plasmid pCMVIE-AKI-DHFR (Whang, Y., Silberklang, M., Morgan,

A., Munshi, S., Lenny, A.B., Ellis, R.W., and Kieff, E. (1987) *J. Virol.*, 61, 1796-1807) by removing an EcoRI fragment containing the DHFR and Neomycin markers. Five copies of the fos promoter serum response element were inserted as described previously (Jones, R.E., Defeo-Jones, D., McAvoy, E.M., Vuocolo, G.A., Wegrzyn, R.J., Haskell, K.M. and Oliff, A. (1991) *Oncogene*, 6, 745-751) to create plasmid pCMV-RE-AKI.

The plasmid pGEM7zf(-)/SEAP was constructed as follows. The SEAP gene was PCR'd, in two segments from a human placenta cDNA library (Clontech) using the following oligos.

10

Sense strand N-terminal SEAP : 5' GAGAGGGAATTCGGGCCCTTCCTGCAT
GCTGCTGCTGCTGCTGCTGCTGGGC 3' (SEQ.ID.NO.:4)

15

Antisense strand N-terminal SEAP: 5' GAGAGAGCTCGAGGTTAACCCGGGT
GCGCGGCGTCGGTGGT 3' (SEQ.ID.NO.:5)

Sense strand C-terminal SEAP: 5' GAGAGAGTCTAGAGTTAACCCGTGGTCC
CCGCGTTGCTTCCT 3' (SEQ.ID.NO.:6)

20

Antisense strand C-terminal SEAP: 5' GAAGAGGAAGCTTGGTACCGCCACTG
GGCTGTAGGTGGTGGCT 3' (SEQ.ID.NO.:7)

The N-terminal oligos (SEQ.ID.NO.: 4 and SEQ.ID.NO.: 5) were used to generate a 1560 bp N-terminal PCR product that contained EcoRI and HpaI restriction sites at the ends. The Antisense N-terminal oligo (SEQ.ID.NO.: 5) introduces an internal translation STOP codon within the SEAP gene along with the HpaI site. The C-terminal oligos (SEQ.ID.NO.: 6 and SEQ.ID.NO.: 7) were used to amplify a 412 bp C-terminal PCR product containing HpaI and HindIII restriction sites. The sense strand C-terminal oligo (SEQ.ID.NO.: 6) introduces the internal STOP codon as well as the HpaI site. Next, the N-terminal amplicon was digested with EcoRI and HpaI while the C-terminal amplicon was digested with HpaI and HindIII. The two fragments comprising each end of the SEAP gene were isolated by electro-phoresing the digest in an agarose gel and isolating the 1560 and 412 base pair fragments. These two fragments were then co-ligated into the vector pGEM7zf

(-) (Promega) which had been restriction digested with EcoRI and HindIII and isolated on an agarose gel. The resulting clone, pGEM7zf(-)/SEAP contains the coding sequence for the SEAP gene from amino acids.

5 Construction of a constitutively expressing SEAP plasmid pCMV-SEAP-A

An expression plasmid constitutively expressing the SEAP protein was created by placing the sequence encoding a truncated SEAP gene downstream of the cytomegalovirus (CMV) IE-1 promoter. The expression plasmid also includes the CMV intron A region 5' to the SEAP gene as well as the 3' untranslated region of the bovine growth hormone gene 3' to the SEAP gene.

The plasmid pCMVIE-AKI-DHFR (Whang, Y., Silberklang, M., Morgan, A., Munshi, S., Lenny, A.B., Ellis, R.W., and Kieff, E. (1987) J. Virol., 61:1796-1807) containing the CMV immediate early promoter was cut with EcoRI generating two fragments. The vector fragment was isolated by agarose electrophoresis and religated. The resulting plasmid is named pCMV-AKI. Next, the cytomegalovirus intron A nucleotide sequence was inserted downstream of the CMV IE1 promoter in pCMV-AKI. The intron A sequence was isolated from a genomic clone bank and subcloned into pBR322 to generate plasmid p16T-286. The intron A sequence was mutated at nucleotide 1856 (nucleotide numbering as in Chapman, B.S., Thayer, R.M., Vincent, K.A. and Haigwood, N.L., Nuc.Acids Res. 19, 3979-3986) to remove a SacI restriction site using site directed mutagenesis. The mutated intron A sequence was PCR'd from the plasmid p16T-287 using the following oligos.

25 Sense strand: 5' GGCAGAGCTCGTTTAGTGAACCGTCAG 3' (SEQ.ID.NO.: 8)

Antisense strand: 5' GAGAGATCTCAAGGACGGTGACTGCAG 3' (SEQ.ID.NO.: 9)

30 These two oligos generate a 991 base pair fragment with a SacI site incorporated by the sense oligo and a Bgl-II fragment incorporated by the antisense oligo. The PCR fragment is trimmed with SacI and Bgl-II and isolated on an agarose gel. The vector pCMV-AKI is cut with SacI and Bgl-II and the larger vector fragment isolated by agarose gel electrophoresis. The two gel isolated fragments are ligated at their respective SacI and Bgl-II sites to create plasmid pCMV-AKI-InA.

The DNA sequence encoding the truncated SEAP gene is inserted into the pCMV-AKI-InA plasmid at the Bgl-II site of the vector. The SEAP gene is cut out of plasmid pGEM7zf(-)/SEAP (described above) using EcoRI and HindIII. The fragment is filled in with Klenow DNA polymerase and the 1970 base pair fragment isolated from the vector fragment by agarose gel electrophoresis. The pCMV-AKI-InA vector is prepared by digesting with Bgl-II and filling in the ends with Klenow DNA polymerase. The final construct is generated by blunt end ligating the SEAP fragment into the pCMV-AKI-InA vector. Transformants were screened for the proper insert and then mapped for restriction fragment orientation. Properly oriented recombinant constructs were sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid, named pCMV-SEAP-A (deposited in the ATCC under Budapest Treaty on August 27, 1998, and designated ATCC), contains a modified SEAP sequence downstream of the cytomegalovirus immediately early promoter IE-1 and intron A sequence and upstream of the bovine growth hormone poly-A sequence. The plasmid expresses SEAP in a constitutive manner when transfected into mammalian cells.

Alternative construction of a constitutively expressing SEAP plasmid pCMV-SEAP-B

An expression plasmid constitutively expressing the SEAP protein can be created by placing the sequence encoding a truncated SEAP gene downstream of the cytomegalovirus (CMV) IE-1 promoter and upstream of the 3' untranslated region of the bovine growth hormone gene.

The plasmid pCMVIE-AKI-DHFR (Whang, Y., Silberklang, M., Morgan, A., Munshi, S., Lenny, A.B., Ellis, R.W., and Kieff, E. (1987) J. Virol., 61:1796-1807) containing the CMV immediate early promoter and bovine growth hormone poly-A sequence can be cut with EcoRI generating two fragments. The vector fragment can be isolated by agarose electrophoresis and religated. The resulting plasmid is named pCMV-AKI. The DNA sequence encoding the truncated SEAP gene can be inserted into the pCMV-AKI plasmid at a unique Bgl-II in the vector. The SEAP gene is cut out of plasmid pGEM7zf(-)/SEAP (described above) using EcoRI and HindIII. The fragments are filled in with Klenow DNA polymerase and the 1970 base pair fragment is isolated from the vector fragment by agarose gel electrophoresis. The pCMV-AKI vector is prepared by digesting with Bgl-II and filling in the ends with Klenow DNA polymerase. The final construct is generated

by blunt end ligating the SEAP fragment into the vector and transforming the ligation reaction into *E. coli* DH5 α cells. Transformants can then be screened for the proper insert and mapped for restriction fragment orientation. Properly oriented recombinant constructs would be sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid, named pCMV-SEAP-B contains a modified SEAP sequence downstream of the cytomegalovirus immediate early promoter, IE1, and upstream of a bovine growth hormone poly-A sequence. The plasmid would express SEAP in a constitutive manner when transfected into mammalian cells.

10 Cloning of a Myristylated viral-H-*ras* expression plasmid pSMS600

A DNA fragment containing viral-H-*ras* can be PCR'd from plasmid "HB-11 (deposited in the ATCC under Budapest Treaty on August 27, 1997, and designated ATCC 209,218) using the following oligos.

15 Sense strand:

5'TCTCCTCGAGGCCACCATGGGGAGTAGCAAGAGCAAGCCTAAGGACCC
CAGCCAGCGCCGGATGACAGAATACAAGCTTGTGGTGG 3'. (SEQ.ID.NO.:
10)

20 Antisense:

5'CACATCTAGATCAGGACAGCACAGACTTGCAGC 3'.
(SEQ.ID.NO.: 11)

A sequence encoding the first 15 aminoacids of the v-src gene,
25 containing a myristylation site, is incorporated into the sense strand oligo. The sense strand oligo also optimizes the 'Kozak' translation initiation sequence immediately 5' to the ATG start site. To prevent prenylation at the viral-*ras* C-terminus, cysteine 186 would be mutated to a serine by substituting a G residue for a C residue in the C-terminal antisense oligo. The PCR primer oligos introduce an XhoI site at the
30 5' end and a XbaI site at the 3' end. The XhoI-XbaI fragment can be ligated into the mammalian expression plasmid pCI (Promega) cut with XhoI and XbaI. This results in a plasmid, pSMS600, in which the recombinant myr-viral-H-*ras* gene is constitutively transcribed from the CMV promoter of the pCI vector.

35 Cloning of a viral-H-*ras*-CVLL expression plasmid pSMS601

A viral-*H-ras* clone with a C-terminal sequence encoding the amino acids CVLL can be cloned from the plasmid "HB-11" by PCR using the following oligos.

5 Sense strand:

5'TCTCCTCGAGGCCACCATGACAGAATACAAGCTTGTGGTGG-3'
(SEQ.ID.NO.: 12)

Antisense strand:

10 5'CACTCTAGACTGGTGTTCAGAGCAGCACACACTTGCAGC-3' (SEQ.ID.NO.: 13)

The sense strand oligo optimizes the 'Kozak' sequence and adds an XhoI site. The antisense strand mutates serine 189 to leucine and adds an XbaI site.

15 The PCR fragment can be trimmed with XhoI and XbaI and ligated into the XhoI-XbaI cut vector pCI (Promega). This results in a plasmid, pSMS601, in which the mutated viral-*H-ras*-CVLL gene is constitutively transcribed from the CMV promoter of the pCI vector.

20 Cloning of cellular-*H-ras*-Leu61 expression plasmid pSMS620

The human cellular-*H-ras* gene can be PCRed from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

Sense strand:

25 5'-GAGAGAATTCGCCACCATGACGGAATATAAGCTGGTGG-3'
(SEQ.ID.NO.: 14)

Antisense strand:

30 5'-GAGAGTCGACGCGTCAGGAGAGCACACACTTGC-3' (SEQ.ID.NO.: 15)

The primers will amplify a c-H-Ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, an EcoRI site at the N-terminus and a Sal I site at the C-terminal end. After trimming the ends of the PCR product with EcoRI and Sal I, the c-H-ras fragment can be ligated into an EcoRI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of glutamine-61

to a leucine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-CCGCCGGCCTGGAGGAGTACAG-3' (SEQ.ID.NO.: 16)

5

After selection and sequencing for the correct nucleotide substitution, the mutated c-H-*ras*-Leu61 can be excised from the pAlter-1 vector, using EcoRI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with EcoRI and Sal I. The new recombinant plasmid, pSMS620, will constitutively transcribe c-H-*ras*-Leu61 from the CMV promoter of the pCI vector.

10

Cloning of a c-N-*ras*-Val-12 expression plasmid pSMS630

The human c-N-*ras* gene can be PCR'd from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

15

Sense strand:

5'-GAGAGAATTTCGCCACCATGACTGAGTACAAACTGGTGG-3'
(SEQ.ID.NO.: 17)

20 Antisense strand:

5'-GAGAGTCGACTTGTTACATCACACACATGGC-3' (SEQ.ID.NO.: 18)

The primers will amplify a c-N-Ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, an EcoRI site at the N-terminus and a Sal I site at the C-terminal end. After trimming the ends of the PCR product with EcoRI and Sal I, the c-N-*ras* fragment can be ligated into an EcoRI-Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of glycine-12 to a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

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5'-GTTGGAGCAGTTGGTGTGGG-3' (SEQ.ID.NO.: 19)

After selection and sequencing for the correct nucleotide substitution, the mutated c-N-*ras*-Val-12 can be excised from the pAlter-1 vector, using EcoRI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested

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with EcoRI and Sal I. The new recombinant plasmid, pSMS630, will constitutively transcribe c-N-*ras*-Val-12 from the CMV promoter of the pCI vector.

Cloning of a c-K4B-*ras*-Val-12 expression plasmid pSMS640

5 The human c-K4B-*ras* gene can be PCR'd from a human cerebral cortex cDNA library (Clontech) using the following oligo-nucleotide primers.

Sense strand:

5'-GAGAGGTACCGCCACCATGACTGAATATAAACTTGTGG-3'

10 (SEQ.ID.NO.: 20)

Antisense strand:

5'-CTCTGTCGACGTATTTACATAATTACACACTTTGTC-3' (SEQ.ID.NO.: 21)

15 The primers will amplify a c-K4B-Ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, a KpnI site at the N-terminus and a Sal I site at the C-terminal end. After trimming the ends of the PCR product with Kpn I and Sal I, the c-K4B-*ras* fragment can be ligated into a KpnI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of cysteine-12 to
20 a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-GTAGTTGGAGCTGTTGGCGTAGGC-3' (SEQ.ID.NO.: 22)

25 After selection and sequencing for the correct nucleotide substitution, the mutated c-K4B-*ras*-Val-12 can be excised from the pAlter-1 vector, using KpnI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with KpnI and Sal I. The new recombinant plasmid will constitutively transcribe c-K4B-*ras*-Val-12 from the CMV promoter of the pCI vector.

30

Cloning of c-K-*ras*4A-Val-12 expression plasmid pSMS650

The human c-K4A-*ras* gene can be PCR'd from a human cerebral cortex cDNA library (Clontech) using the following oligo-nucleotide primers.

35 Sense strand:

5'-GAGAGGTACCGCCACCATGACTGAATATAAACTTGTGG-3'
(SEQ.ID.NO.: 23)

Antisense strand:

5 5'-CTCTGTCGACAGATTACATTATAATGCATTTTTTAATTTTCACAC-3'
(SEQ.ID.NO.: 24)

The primers will amplify a c-K4A-Ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, a KpnI site at the N-terminus and a Sal I site at the C-terminal end. After trimming the ends of the PCR product with Kpn I and Sal I, the c-K-ras4A fragment can be ligated into a KpnI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of cysteine-12 to a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

15 5'-GTAGTTGGAGCTGTTGGCGTAGGC-3' (SEQ.ID.NO.: 25)

After selection and sequencing for the correct nucleotide substitution, the mutated c-K4A-ras-Val-12 can be excised from the pAlter-1 vector, using KpnI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with KpnI and Sal I. The new recombinant plasmid, pSMS650, will constitutively transcribe c-K4A-ras-Val-12 from the CMV promoter of the pCI vector.

25 SEAP assay

Human C33A cells (human epithelial carcinoma - ATTC collection) are seeded in 10cm tissue culture plates in DMEM + 10% fetal calf serum + 1X Pen/Strep + 1X glutamine + 1X NEAA. Cells are grown at 37°C in a 5% CO₂ atmosphere until they reach 50-80% of confluency.

30 The transient transfection is performed by the CaPO₄ method (Sambrook et al., 1989). Thus, expression plasmids for H-ras, N-ras, K-ras, Myr-ras or H-ras-CVLL are co-precipitated with the DSE-SEAP reporter construct. (A ras expression plasmid is not included when the cell is transfected with the pCMV-SEAP plasmid.) For 10 cm plates 600 µl of CaCl₂-DNA solution is added dropwise
35 while vortexing to 600 µl of 2X HBS buffer to give 1.2 ml of precipitate solution

(see recipes below). This is allowed to sit at room temperature for 20 to 30 minutes. While the precipitate is forming, the media on the C33A cells is replaced with DMEM (minus phenol red; Gibco cat. No. 31053-028)+ 0.5% charcoal stripped calf serum + 1X (Pen/Strep, Glutamine and nonessential aminoacids). The CaPO₄-DNA precipitate is added dropwise to the cells and the plate rocked gently to distribute. DNA uptake is allowed to proceed for 5-6 hrs at 37°C under a 5% CO₂ atmosphere.

Following the DNA incubation period, the cells are washed with PBS and trypsinized with 1ml of 0.05% trypsin. The 1 ml of trypsinized cells is diluted into 10 ml of phenol red free DMEM + 0.2% charcoal stripped calf serum + 1X (Pen/Strep, Glutamine and NEAA). Transfected cells are plated in a 96 well micro-titer plate (100 µl/well) to which drug, diluted in media, has already been added in a volume of 100 µl. The final volume per well is 200 µl with each drug concentration repeated in triplicate over a range of half-log steps.

Incubation of cells and drugs is for 36 hours at 37°C under CO₂. At the end of the incubation period, cells are examined micro-scopically for evidence of cell distress. Next, 100 µl of media containing the secreted alkaline phosphatase is removed from each well and transferred to a microtube array for heat treatment at 65°C for 1 hour to inactivate endogenous alkaline phosphatases (but not the heat stable secreted phosphatase).

The heat treated media is assayed for alkaline phosphatase by a luminescence assay using the luminescence reagent CSPD® (Tropix, Bedford, Mass.). A volume of 50 µl media is combined with 200 µl of CSPD cocktail and incubated for 60 minutes at room temperature. Luminescence is monitored using an ML2200 microplate luminometer (Dynatech). Luminescence reflects the level of activation of the fos reporter construct stimulated by the transiently expressed protein.

DNA-CaPO₄ precipitate for 10cm. plate of cells

Ras expression plasmid (1 µg/µl)	10 µl
30 DSE-SEAP Plasmid (1 µg/µl)	2 µl
Sheared Calf Thymus DNA (1 µg/µl)	8 µl
2M CaCl ₂	74 µl
dH ₂ O	506 µl

2X HBS Buffer

280mM NaCl

10mM KCl

1.5mM Na₂HPO₄ 2H₂O

5 12mM dextrose

50mM HEPES

Final pH = 7.05

Luminescence Buffer (26ml)

10 Assay Buffer 20ml

Emerald Reagent™ (Tropix) 2.5ml

100mM homoarginine 2.5ml

CSPD Reagent® (Tropix) 1.0ml

15 Assay BufferAdd 0.05M Na₂CO₃ to 0.05M NaHCO₃ to obtain pH 9.5.Make 1mM in MgCl₂

EXAMPLE 10

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The processing assays employed are modifications of that described by DeClue et al [Cancer Research 51, 712-717, 1991].

K4B-Ras processing inhibition assay

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PSN-1 (human pancreatic carcinoma) or viral-K4B-ras-transformed

Rat1 cells are used for analysis of protein processing. Subconfluent cells in 100 mm dishes are fed with 3.5 ml of media (methionine-free RPMI supplemented with 2% fetal bovine serum or cysteine-free/methionine-free DMEM supplemented with 0.035 ml of 200 mM glutamine (Gibco), 2% fetal bovine serum, respectively) containing the desired concentration of test compound, lovastatin or solvent alone. Cells treated with lovastatin (5-10 μM), a compound that blocks Ras processing in cells by inhibiting a rate-limiting step in the isoprenoid biosynthetic pathway, serve as a positive control. Test compounds are prepared as 1000x concentrated solutions in DMSO to yield a final solvent concentration of 0.1%. Following incubation at

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37°C for two hours 204 $\mu\text{Ci/ml}$ [^{35}S]Pro-Mix (Amersham, cell labeling grade) is added.

After introducing the label amino acid mixture, the cells are incubated at 37°C for an additional period of time (typically 6 to 24 hours). The media is then removed and the cells are washed once with cold PBS. The cells are scraped into 1 ml of cold PBS, collected by centrifugation (10,000 x g for 10 sec at room temperature), and lysed by vortexing in 1 ml of lysis buffer (1% Nonidet P-40, 20 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% deoxycholate, 0.1% SDS, 1 mM DTT, 10 $\mu\text{g/ml}$ AEBSF, 10 $\mu\text{g/ml}$ aprotinin, 2 $\mu\text{g/ml}$ leupeptin and 2 $\mu\text{g/ml}$ antipain). The lysate is then centrifuged at 15,000 x g for 10 min at 4°C and the supernatant saved.

For immunoprecipitation of Ki4B-Ras, samples of lysate supernatant containing equal amounts of protein are utilized. Protein concentration is determined by the bradford method utilizing bovine serum albumin as a standard. The appropriate volume of lysate is brought to 1 ml with lysis buffer lacking DTT and 8 μg of the pan Ras monoclonal antibody, Y13-259, added. The protein/antibody mixture is incubated on ice at 4°C for 24 hours. The immune complex is collected on pansorbin (Calbiochem) coated with rabbit antiserum to rat IgG (Cappel) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in 100 μl elution buffer (10 mM Tris pH 7.4, 1% SDS). The Ras is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation (15,000 x g for 30 sec. at room temperature).

The supernatant is added to 1 ml of Dilution Buffer (0.1% Triton X-100, 5 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.4) with 2 μg Kirsten-ras specific monoclonal antibody, c-K-ras Ab-1 (Calbiochem). The second protein/antibody mixture is incubated on ice at 4°C for 1-2 hours. The immune complex is collected on pansorbin (Calbiochem) coated with rabbit antiserum to rat IgG (Cappel) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in Laemmli sample buffer. The Ras is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation. The supernatant is subjected to SDS-PAGE on a 12% acrylamide gel (bis-acrylamide:acrylamide, 1:100), and the Ras visualized by fluorography.

hDJ Processing Inhibition Assay

PSN-1 cells are seeded in 24-well assay plates. For each compound to be tested, the cells are treated with a minimum of seven concentrations in half-log steps. The final solvent (DMSO) concentration is 0.1%. A vehicle-only control is included on each assay plate. The cells are treated for 24 hours at 37°C / 5% CO₂.

The growth media is then aspirated and the samples are washed with PBS. The cells are lysed with SDS-PAGE sample buffer containing 5% 2-mercaptoethanol and heated to 95°C for 5 minutes. After cooling on ice for 10 minutes, a mixture of nucleases is added to reduce viscosity of the samples.

The plates are incubated on ice for another 10 minutes. The samples are loaded onto pre-cast 8% acrylamide gels and electrophoresed at 15 mA/gel for 3-4 hours. The samples are then transferred from the gels to PVDF membranes by Western blotting.

The membranes are blocked for at least 1 hour in buffer containing 2% nonfat dry milk. The membranes are then treated with a monoclonal antibody to hDJ-2 (Neomarkers Cat. # MS-225), washed, and treated with an alkaline phosphatase-conjugated secondary antibody. The membranes are then treated with a fluorescent detection reagent and scanned on a phosphorimager.

For each sample, the percent of total signal corresponding to the unprenylated species of hDJ (the slower-migrating species) is calculated by densitometry. Dose-response curves and EC₅₀ values are generated using 4-parameter curve fits in SigmaPlot software.

25 EXAMPLE 11

Rap1 processing inhibition assay

Protocol A:

30 Cells are labeled, incubated and lysed as described in Example 10.

For immunoprecipitation of Rap1, samples of lysate supernatant containing equal amounts of protein are utilized. Protein concentration is determined by the Bradford method utilizing bovine serum albumin as a standard. The appropriate volume of lysate is brought to 1 ml with lysis buffer lacking DTT and 2 µg of

the Rap1 antibody, Rap1/Krev1 (121) (Santa Cruz Biotech), is added. The protein/antibody mixture is incubated on ice at 4°C for 1 hour. The immune complex is collected on pansorbin (Calbiochem) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in 100 µl elution buffer (10 mM Tris pH 7.4, 1% SDS). The Rap1 is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation (15,000 x g for 30 sec. at room temperature).

The supernatant is added to 1 ml of Dilution Buffer (0.1% Triton X-100, 5 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.4) with 2 µg Rap1 antibody, Rap1/Krev1 (121) (Santa Cruz Biotech). The second protein/antibody mixture is incubated on ice at 4°C for 1-2 hours. The immune complex is collected on pansorbin (Calbiochem) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in Laemmli sample buffer. The Rap1 is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation. The supernatant is subjected to SDS-PAGE on a 12% acrylamide gel (bis-acrylamide:acrylamide, 1:100), and the Rap1 visualized by fluorography.

Protocol B:

PSN-1 cells are passaged every 3-4 days in 10cm plates, splitting near-confluent plates 1:20 and 1:40. The day before the assay is set up, 5x 10⁶ cells are plated on 15cm plates to ensure the same stage of confluency in each assay. The media for these cells is RPMI 1640 (Gibco), with 15% fetal bovine serum and 1x Pen/Strep antibiotic mix.

The day of the assay, cells are collected from the 15 cm plates by trypsinization and diluted to 400,000 cells/ml in media. 0.5ml of these diluted cells are added to each well of 24-well plates, for a final cell number of 200,000 per well. The cells are then grown at 37°C overnight.

The compounds to be assayed are diluted in DMSO in 1/2-log dilutions. The range of final concentrations to be assayed is generally 0.1-100 µM. Four concentrations per compound is typical. The compounds are diluted so that each concentration is 1000x of the final concentration (i.e., for a 10 µM data point, a 10 mM stock of the compound is needed).

2 μ L of each 1000x compound stock is diluted into 1 ml media to produce a 2X stock of compound. A vehicle control solution (2 μ L DMSO to 1ml media), is utilized. 0.5 ml of the 2X stocks of compound are added to the cells.

After 24 hours, the media is aspirated from the assay plates. Each
5 well is rinsed with 1ml PBS, and the PBS is aspirated. 180 μ L SDS-PAGE sample buffer (Novex) containing 5% 2-mercapto-ethanol is added to each well. The plates are heated to 100°C for 5 minutes using a heat block containing an adapter for assay plates. The plates are placed on ice. After 10 minutes, 20 μ L of an RNase/DNase mix is added per well. This mix is 1mg/ml DNaseI (Worthington Enzymes), 0.25
10 mg/ml Rnase A (Worthington Enzymes), 0.5 M Tris-HCl pH 8.0 and 50 mM MgCl₂. The plate is left on ice for 10 minutes. Samples are then either loaded on the gel, or stored at -70°C until use.

Each assay plate (usually 3 compounds, each in 4-point titrations, plus controls) requires one 15-well 14% Novex gel. 25 μ L of each sample is loaded onto
15 the gel. The gel is run at 15 mA for about 3.5 hours. It is important to run the gel far enough so that there will be adequate separation between 21 kd (Rap1) and 29kd (Rab6).

The gels are then transferred to Novex pre-cut PVDF membranes for 1.5 hours at 30V (constant voltage). Immediately after transferring, the membranes
20 are blocked overnight in 20ml Western blocking buffer (2% nonfat dry milk in Western wash buffer (PBS + 0.1% Tween-20). If blocked over the weekend, 0.02% sodium azide is added. The membranes are blocked at 4°C with slow rocking.

The blocking solution is discarded and 20ml fresh blocking solution containing the anti Rap1a antibody (Santa Cruz Biochemical SC1482) at 1:1000
25 (diluted in Western blocking buffer) and the anti Rab6 antibody (Santa Cruz Biochemical SC310) at 1:5000 (diluted in Western blocking buffer) are added. The membranes are incubated at room temperature for 1 hour with mild rocking. The blocking solution is then discarded and the membrane is washed 3 times with Western wash buffer for 15 minutes per wash. 20ml blocking solution containing
30 1:1000 (diluted in Western blocking buffer) each of two alkaline phosphatase conjugated antibodies (Alkaline phosphatase conjugated Anti-goat IgG and Alkaline phosphatase conjugated anti-rabbit IgG [Santa Cruz Biochemical]) is then added. The membrane is incubated for one hour and washed 3x as above.

About 2 ml per gel of the Amersham ECF detection reagent is placed
35 on an overhead transparency (ECF) and the PVDF membranes are placed face-down

onto the detection reagent. This is incubated for one minute, then the membrane is placed onto a fresh transparency sheet.

The developed transparency sheet is scanned on a phosphorimager and the Rap1a Minimum Inhibitory Concentration is determined from the lowest concentration of compound that produces a detectable Rap1a Western signal. The Rap1a antibody used recognizes only unprenylated/unprocessed Rap1a, so that the presence of a detectable Rap1a Western signal is indicative of inhibition of Rap1a prenylation.

10 Protocol C:

This protocol allows the determination of an EC₅₀ for inhibition of processing of Rap1a. The assay is run as described in Protocol B with the following modifications. 20 μ l of sample is run on pre-cast 10-20% gradient acrylamide mini gels (Novex Inc.) at 15 mA/gel for 2.5-3 hours. Prenylated and unprenylated forms of Rap1a are detected by blotting with a polyclonal antibody (Rap1/Krev-1 Ab#121; Santa Cruz Research Products #sc-65), followed by an alkaline phosphatase-conjugated anti-rabbit IgG antibody. The percentage of unprenylated Rap1a relative to the total amount of Rap1a is determined by peak integration using ImagequantTM software (Molecular Dynamics). Unprenylated Rap1a is distinguished from prenylated protein by virtue of the greater apparent molecular weight of the prenylated protein. Dose-response curves and EC₅₀ values are generated using 4-parameter curve fits in SigmaPlot software.

EXAMPLE 12

25 *In vivo* tumor growth inhibition assay (nude mouse)

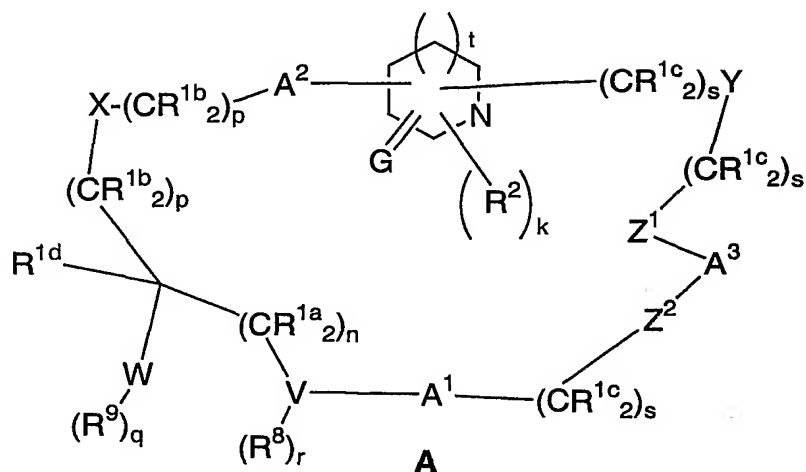
In vivo efficacy as an inhibitor of the growth of cancer cells may be confirmed by several protocols well known in the art. Examples of such *in vivo* efficacy studies are described by N. E. Kohl et al. (Nature Medicine, 1:792-797 (1995)) and N. E. Kohl et al. (Proc. Nat. Acad. Sci. U.S.A., 91:9141-9145 (1994)).

Rodent fibroblasts transformed with oncogenically mutated human Ha-ras or Ki-ras (10⁶ cells/animal in 1 ml of DMEM salts) are injected subcutaneously into the left flank of 8-12 week old female nude mice (Harlan) on day 0. The mice in each oncogene group are randomly assigned to a vehicle or compound treatment group. Animals are dosed subcutaneously starting on day

1 and daily for the duration of the experiment. Alternatively, the farnesyl-protein transferase inhibitor may be administered by a continuous infusion pump. Compound or vehicle is delivered in a total volume of 0.1 ml. Tumors are excised and weighed when all of the vehicle-treated animals exhibited lesions of 0.5-1.0 cm in diameter,
5 typically 11-15 days after the cells were injected. The average weight of the tumors in each treatment group for each cell line is calculated.

WHAT IS CLAIMED IS:

1. A compound of the formula A:



5 wherein:

R^{1a}, R^{1b}, R^{1c} and R^{1d} are independently selected from:

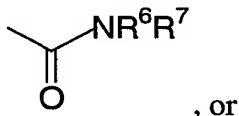
- a) hydrogen,
- b) unsubstituted or substituted aryl,
- c) unsubstituted or substituted heterocycle,
- 10 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- e) R¹⁰O-,
- f) R¹¹S(O)_m-,
- g) R¹⁰C(O)NR¹⁰-,
- h) (R¹⁰)₂NC(O)-,
- 15 i) CN,
- j) NO₂,
- k) R¹⁰C(O)-,
- l) R¹⁰OC(O)-,
- m) -N(R¹⁰)₂,
- 20 n) R¹¹OC(O)NR¹⁰-,
- o) unsubstituted or substituted C₁-C₆ alkyl,
- p) unsubstituted or substituted C₂-C₆ alkenyl, or
- q) unsubstituted or substituted C₂-C₆ alkynyl,

wherein the substituent on the substituted group is selected from unsubstituted or substituted C₁-C₆ alkyl, unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, unsubstituted or substituted C₃-C₁₀ cycloalkyl, unsubstituted or unsubstituted C₂-C₆ alkenyl, unsubstituted or substituted C₂-C₆ alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, and R¹¹OC(O)-NR¹⁰-,

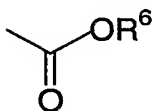
or two R^{1a}s, two R^{1b}s or two R^{1c}s, on the same carbon atom may be combined to form -(CH₂)_u-;

R² is independently selected from

- a) hydrogen,
- b) unsubstituted or substituted C₁-8 alkyl,
- c) unsubstituted or substituted C₂-8 alkenyl,
- d) unsubstituted or substituted C₂-8 alkynyl,
- e) unsubstituted or substituted aryl,
- f) unsubstituted or substituted heterocycle,
- g)

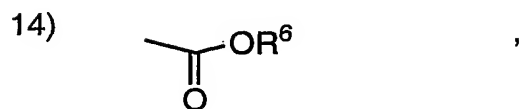
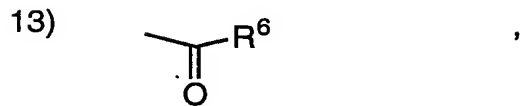
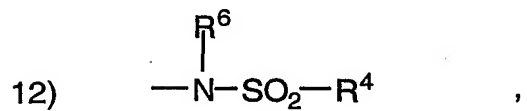
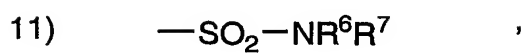
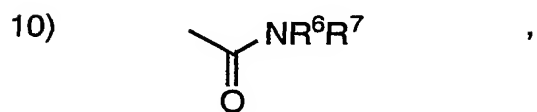
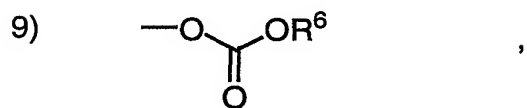
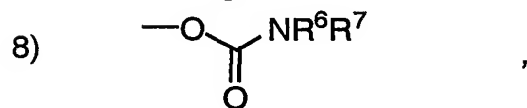
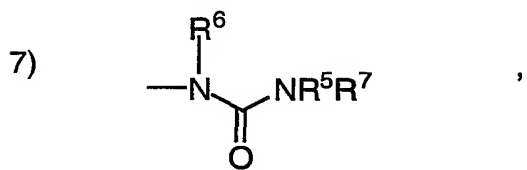
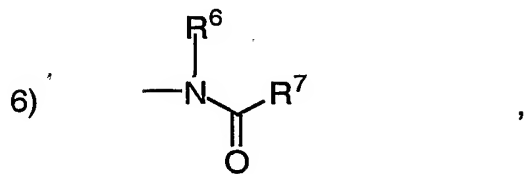


h)

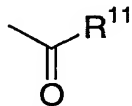


wherein the substituted group is substituted with one or more of:

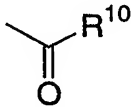
- 1) aryl or heterocycle, unsubstituted or substituted with:
 - a) C₁-4 alkyl,
 - b) (CH₂)_pOR⁶,
 - c) (CH₂)_pNR⁶R⁷,
 - d) halogen,
 - e) CN,
- 2) C₃-6 cycloalkyl,
- 3) OR⁶,
- 4) SR⁴, S(O)R⁴, SO₂R⁴,



R⁴ is selected from C₁₋₆ alkyl, C₃₋₆ cycloalkyl, heterocycle, aryl, unsubstituted or substituted with:

- 5
- 1) C₁₋₆ alkoxy,
 - 2) aryl or heterocycle,
 - 3) halogen,
 - 4) HO,
 - 5)
- 
- 10
- 6) SO₂R¹¹,
 - 7) N(R¹⁰)₂, or
 - 8) C₁₋₄ perfluoroalkyl;

R⁵, R⁶ and R⁷ are independently selected from:

- 15
- a) hydrogen,
 - b) R¹⁰C(O)-,
 - c) R¹⁰OC(O)-, or
 - d) C_{1-C6} alkyl, C_{2-C6} alkenyl, C_{2-C6} alkynyl, C₃₋₆ cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with one or more substituents selected
- 20
- from:
- 1) unsubstituted or substituted C_{1-C6} alkyl,
 - 2) R¹⁰O-,
 - 3) unsubstituted or substituted aryl or heterocycle,
 - 4) halogen,
 - 5) R¹⁰C(O)NR¹⁰-,
 - 6)
- 
- 25
- 7) SO₂R¹¹,
 - 8) N(R¹⁰)₂,
 - 9) C₃₋₆ cycloalkyl,
- 30

- 5
- 10) C₁-C₆ perfluoroalkyl,
 - 11) R¹⁰OC(O)-,
 - 12) R¹¹OC(O)NR¹⁰-,
 - 13) CN, and
 - 14) NO₂; or

R⁶ and R⁷ may be joined in a ring; and independently,

R⁵ and R⁷ may be joined in a ring;

10

R⁸ is independently selected from:

- a) hydrogen,
- b) unsubstituted or substituted aryl,
- c) unsubstituted or substituted heterocycle,
- 15 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- e) unsubstituted or substituted C₂-C₆ alkenyl,
- f) unsubstituted or substituted C₂-C₆ alkynyl,
- g) C₁-C₆ perfluoroalkyl,
- h) halo,
- 20 i) R¹⁰O-,
- j) R¹¹S(O)_m-,
- k) R¹⁰C(O)NR¹⁰-,
- l) (R¹⁰)₂NC(O)-,
- m) CN,
- 25 n) NO₂,
- o) R¹⁰C(O)-,
- p) R¹⁰OC(O)-,
- q) -N(R¹⁰)₂,
- r) R¹¹OC(O)NR¹⁰-, and
- 30 s) unsubstituted or substituted C₁-C₆ alkyl, optionally substituted by unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, unsubstituted or substituted C₃-C₁₀ cycloalkyl, unsubstituted or substituted C₂-C₆ alkenyl, unsubstituted or substituted C₂-C₆ alkynyl, C₁-C₆ perfluoroalkyl, F, Cl, Br, R¹⁰O-,

$R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, $(R^{10})_2NC(O)-$, CN, $R^{10}C(O)-$,
 $R^{10}OC(O)-$, $-N(R^{10})_2$, or $R^{10}OC(O)NR^{10}-$;

R^9 is selected from:

- 5 a) hydrogen,
- b) unsubstituted or substituted C_2-C_6 alkenyl,
- c) unsubstituted or substituted C_2-C_6 alkynyl,
- d) C_1-C_6 perfluoroalkyl,
- e) halo,
- 10 f) $R^{10}O-$,
- g) $R^{11}S(O)_m-$,
- h) $R^{10}C(O)NR^{10}-$,
- i) $(R^{10})_2NC(O)-$,
- j) CN,
- 15 k) NO_2 ,
- l) $R^{10}C(O)-$,
- m) $R^{10}OC(O)-$,
- n) $-N(R^{10})_2$,
- o) $R^{11}OC(O)NR^{10}-$, or
- 20 p) unsubstituted or substituted C_1-C_6 alkyl optionally substituted by aryl,
 C_3-C_{10} cycloalkyl, C_1-C_6 perfluoroalkyl, F, Cl, Br, $R^{10}O-$,
 $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, $(R^{10})_2NC(O)-$, CN, $R^{10}C(O)-$,
 $R^{10}OC(O)-$, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$;

25 R^{10} is independently selected from

- a) hydrogen,
- b) unsubstituted or substituted C_1-C_6 alkyl,
- c) unsubstituted or substituted C_1-C_3 perfluoroalkyl,
- d) unsubstituted or substituted benzyl,
- 30 e) unsubstituted or substituted aryl, or
- f) unsubstituted or substituted heterocycle;

R^{11} is independently selected from

- a) unsubstituted or substituted C_1-C_6 alkyl,

- b) unsubstituted or substituted aryl, or
- c) unsubstituted or substituted heterocycle;

A¹ and A³ are independently selected from

- 5 a) a bond,
- b) -C(O)-,
- c) -C(O)NR¹⁰-,
- d) -NR¹⁰C(O)-,
- e) O,
- 10 f) -N(R¹⁰)-,
- g) -S(O)₂N(R¹⁰)-,
- h) -N(R¹⁰)S(O)₂-, or
- i) S(O)_m;

15 A² is selected from

- a) a bond,
- b) -C(O)-,
- c) -C(O)NR¹⁰-,
- d) -NR¹⁰C(O)-,
- 20 e) O,
- f) -N(R¹⁰)-,
- g) -S(O)₂N(R¹⁰)-,
- h) -N(R¹⁰)S(O)₂-,
- i) -NR¹⁰C(O)NR¹⁰-, or
- 25 j) S(O)_m;

G is selected from H₂ or O;

V is selected from:

- 30 a) heterocycle, or
- b) aryl;

W is a heterocycle;

35 X is selected from:

- 5
- a) a bond,
 - b) -C(O)-,
 - c) -C(O)NR¹⁰-,
 - d) -NR¹⁰C(O)-,
 - e) -NR¹⁰C(O)-O-,
 - f) -O-C(O)NR¹⁰-,
 - g) -NR¹⁰C(O)NR¹⁰-,
 - h) O,
 - i) -N(R¹⁰)-,
 - 10 j) -S(O)₂N(R¹⁰)-,
 - k) -N(R¹⁰)S(O)₂-, or
 - l) S(O)_m;

Y is selected from

- 15
- a) a bond,
 - b) -C(=O)-,
 - c) -C(O)NR¹⁰-,
 - d) -C(O)O-, or
 - e) -S(=O)_m;

20

Z¹ is selected from unsubstituted or substituted aryl and unsubstituted or substituted heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with one or more of:

- 25
- 1) C₁₋₈ alkyl, C₂₋₈ alkenyl or C₂₋₈ alkynyl, unsubstituted or substituted with:
 - a) C₁₋₄ alkoxy,
 - b) NR⁶R⁷,
 - c) C₃₋₆ cycloalkyl,
 - d) aryl or heterocycle,
 - 30 e) HO,
 - f) -S(O)_mR⁴,
 - g) -C(O)NR⁶R⁷, or
 - h) C₁₋₄ perfluoroalkyl;
 - 2) substituted or unsubstituted aryl or substituted or unsubstituted heterocycle,
- 35

- 5
- 3) halogen,
 - 4) OR⁶,
 - 5) NR⁶R⁷,
 - 6) CN,
 - 7) NO₂,
 - 8) CF₃;
 - 9) -S(O)_mR⁴,
 - 10) -OS(O)₂R⁴,
 - 11) -C(O)NR⁶R⁷,
 - 10 12) -C(O)OR⁶, or
 - 13) C₃-C₆ cycloalkyl;

15 Z² is selected from a bond, unsubstituted or substituted aryl and unsubstituted or substituted heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with one or more of:

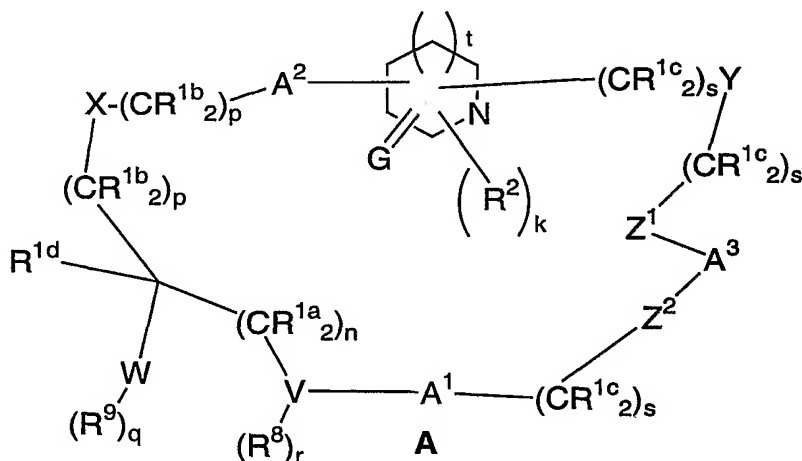
- 1) C₁₋₈ alkyl, C₂₋₈ alkenyl or C₂₋₈ alkynyl, unsubstituted or substituted with:
 - a) C₁₋₄ alkoxy,
 - b) NR⁶R⁷,
 - 20 c) C₃₋₆ cycloalkyl,
 - d) aryl or heterocycle,
 - e) HO,
 - f) -S(O)_mR⁴,
 - g) -C(O)NR⁶R⁷, or
 - 25 h) C₁₋₄ perfluoroalkyl;
- 2) substituted or unsubstituted aryl or substituted or unsubstituted heterocycle,
- 3) halogen,
- 4) OR⁶,
- 30 5) NR⁶R⁷,
- 6) CN,
- 7) NO₂,
- 8) CF₃,
- 9) -S(O)_mR⁴,
- 35 10) -OS(O)₂R⁴,

- 11) $-C(O)NR^6R^7$,
- 12) $-C(O)OR^6$, or
- 13) C_3-C_6 cycloalkyl;

- | | | |
|----|--------------------|---------------------|
| 5 | k is | 0, 1, 2, 3 or 4; |
| | m is | 0, 1 or 2; |
| | n is | 0, 1, 2, 3, 4 or 5; |
| | p is independently | 0, 1, 2, 3, 4 or 5; |
| | q is | 1, 2 or 3; |
| 10 | r is | 0, 1, 2, 3, 4 or 5; |
| | s is independently | 0, 1, 2, 3, 4 or 5; |
| | t is | 0 or 1; and |
| | u is independently | 2, 3, 4, 5 or 6; |

- 15 or a pharmaceutically acceptable salt or stereoisomer thereof.

2. The compound, according to Claim 1, of formula A:



wherein:

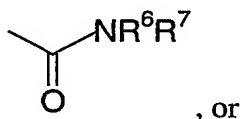
- 20 R1a, R1b, R1c and R1d are independently selected from:
- a) hydrogen,
 - b) unsubstituted or substituted aryl,
 - c) unsubstituted or substituted heterocycle,
 - d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,

- e) $R^{10}O-$,
 f) $R^{11}S(O)_m-$,
 g) $R^{10}C(O)NR^{10}-$,
 h) $(R^{10})_2NC(O)-$,
 5 i) CN ,
 j) NO_2 ,
 k) $R^{10}C(O)-$,
 l) $R^{10}OC(O)-$,
 m) $-N(R^{10})_2$,
 10 n) $R^{11}OC(O)NR^{10}-$,
 o) unsubstituted or substituted C_1-C_6 alkyl,
 p) unsubstituted or substituted C_2-C_6 alkenyl, or
 q) unsubstituted or substituted C_2-C_6 alkynyl,
 wherein the substituent on the substituted group is selected from unsubstituted
 15 or substituted C_1-C_6 alkyl, unsubstituted or substituted aryl,
 unsubstituted or substituted heterocycle, unsubstituted or substituted
 C_3-C_{10} cycloalkyl, unsubstituted or substituted C_2-C_6 alkenyl,
 unsubstituted or substituted C_2-C_6 alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$,
 $R^{10}C(O)NR^{10}-$, $(R^{10})_2NC(O)-$, CN , $R^{10}C(O)-$, $R^{10}OC(O)-$,
 20 $-N(R^{10})_2$, and $R^{11}OC(O)NR^{10}-$,

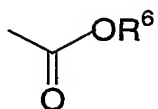
or two R^{1a} s, two R^{1b} s or two R^{1c} s, on the same carbon atom may be combined to form $-(CH_2)_u-$;

25 R^2 is independently selected from

- a) hydrogen,
 b) unsubstituted or substituted C_{1-8} alkyl,
 c) unsubstituted or substituted C_{2-8} alkenyl,
 d) unsubstituted or substituted C_{2-8} alkynyl,
 30 e) unsubstituted or substituted aryl,
 f) unsubstituted or substituted heterocycle,
 g)



h)



wherein the substituted group is substituted with one or more of:

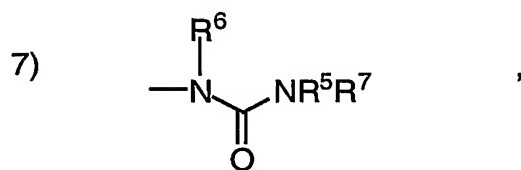
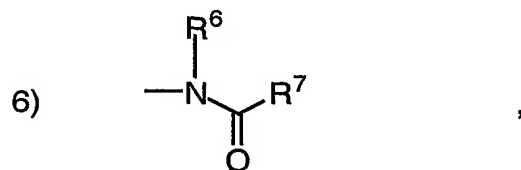
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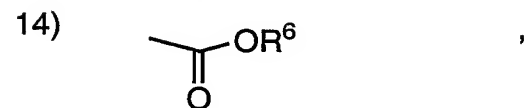
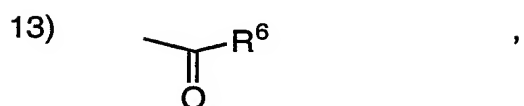
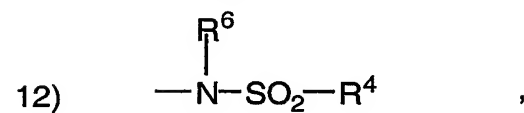
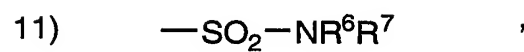
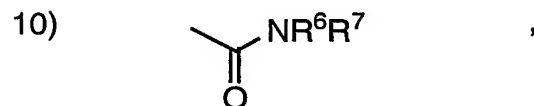
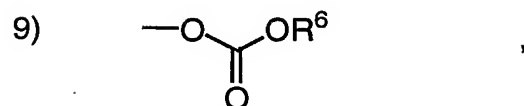
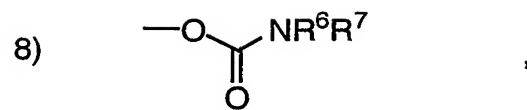
1) aryl or heterocycle, unsubstituted or substituted with:

- a) C₁₋₄ alkyl,
- b) (CH₂)_pOR⁶,
- c) (CH₂)_pNR⁶R⁷,
- d) halogen,
- e) CN,

10

- 2) C₃₋₆ cycloalkyl,
- 3) OR⁶,
- 4) SR⁴, S(O)R⁴, SO₂R⁴,

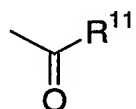




5 R^4 is selected from C_{1-4} alkyl, C_{3-6} cycloalkyl, heterocycle, aryl, unsubstituted or substituted with:

- 1) C_{1-4} alkoxy,
- 2) aryl or heterocycle,
- 3) halogen,
- 4) HO ,
- 5)

10



- 6) SO_2R^{11} ,
- 7) $\text{N}(\text{R}^{10})_2$, or
- 8) C_{1-4} perfluoroalkyl;

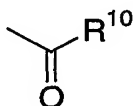
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R^5 , R^6 and R^7 are independently selected from:

- a) hydrogen,
- b) $\text{R}^{10}\text{C}(\text{O})-$,
- c) $\text{R}^{10}\text{OC}(\text{O})-$, or
- 10 d) C_{1-6} alkyl, $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl, $\text{C}_3\text{-6}$ cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with one or more substituents selected from:

15

- 1) $\text{R}^{10}\text{O}-$,
- 2) aryl or heterocycle,
- 3) halogen,
- 4) $\text{R}^{10}\text{C}(\text{O})\text{NR}^{10}-$,
- 5)



20

- 6) SO_2R^{11} ,
- 7) $\text{N}(\text{R}^{10})_2$,
- 8) C_{3-6} cycloalkyl,
- 9) C_{1-6} perfluoroalkyl,
- 25 10) $\text{R}^{10}\text{OC}(\text{O})-$,
- 11) $\text{R}^{11}\text{OC}(\text{O})\text{NR}^{10}-$,
- 12) CN , and
- 13) NO_2 ; or

30 R^6 and R^7 may be joined in a ring; and independently,

R^5 and R^7 may be joined in a ring;

R⁸ is independently selected from:

- a) hydrogen,
- b) unsubstituted or substituted aryl,
- c) unsubstituted or substituted heterocycle,
- 5 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- e) unsubstituted or substituted C₂-C₆ alkenyl,
- f) unsubstituted or substituted C₂-C₆ alkynyl,
- g) C₁-C₆ perfluoroalkyl,
- h) halo,
- 10 i) R¹⁰O-,
- j) R¹¹S(O)_m-,
- k) R¹⁰C(O)NR¹⁰-,
- l) (R¹⁰)₂NC(O)-,
- m) CN,
- 15 n) NO₂,
- o) R¹⁰C(O)-,
- p) R¹⁰OC(O)-,
- q) -N(R¹⁰)₂,
- r) R¹¹OC(O)NR¹⁰-, and
- 20 s) unsubstituted or substituted C₁-C₆ alkyl, optionally substituted by unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹⁰OC(O)NR¹⁰-;
- 25

R⁹ is selected from:

- a) hydrogen,
- b) unsubstituted or substituted C₂-C₆ alkenyl,
- 30 c) unsubstituted or substituted C₂-C₆ alkynyl,
- d) C₁-C₆ perfluoroalkyl,
- e) halo,
- f) R¹⁰O-,
- g) R¹¹S(O)_m-,

- h) $R^{10}C(O)NR^{10}-$,
 i) $(R^{10})_2NC(O)-$,
 j) CN,
 k) NO_2 ,
 5 l) $R^{10}C(O)-$,
 m) $R^{10}OC(O)-$,
 n) $-N(R^{10})_2$,
 o) $R^{11}OC(O)NR^{10}-$, or
 10 p) unsubstituted or substituted C_1-C_6 alkyl optionally substituted by
 C_1-C_6 perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, $(R^{10})_2NC(O)-$, CN, $R^{10}C(O)-$, $R^{10}OC(O)-$, $-N(R^{10})_2$,
 or $R^{11}OC(O)NR^{10}-$;

R^{10} is independently selected from

- 15 a) hydrogen,
 b) unsubstituted or substituted C_1-C_6 alkyl,
 c) unsubstituted or substituted C_1-C_3 perfluoroalkyl,
 d) unsubstituted or substituted benzyl,
 e) unsubstituted or substituted aryl, or
 20 f) unsubstituted or substituted heterocycle;

R^{11} is independently selected from

- a) unsubstituted or substituted C_1-C_6 alkyl,
 b) unsubstituted or substituted aryl, or
 25 c) unsubstituted or substituted heterocycle;

A^1 and A^3 are independently selected from

- a) a bond,
 b) $-C(O)-$,
 30 c) $-C(O)NR^{10}-$,
 d) $-NR^{10}C(O)-$,
 e) O,
 f) $-N(R^{10})-$,
 g) $-S(O)_2N(R^{10})-$,

- h) $-N(R^{10})S(O)_2-$, or
- i) $S(O)_m$;

A² is selected from

- 5 a) a bond,
- b) $-C(O)-$,
- c) $-C(O)NR^{10}-$,
- d) $-NR^{10}C(O)-$,
- e) O,
- 10 f) $-N(R^{10})-$,
- g) $-S(O)_2N(R^{10})-$,
- h) $-N(R^{10})S(O)_2-$,
- i) $-NR^{10}C(O)NR^{10}-$, or
- j) $S(O)_m$;

15

G is selected from H₂ or O;

V is selected from pyridyl or phenyl;

20 W is a heterocycle;

X is selected from:

- a) a bond,
- b) $-C(O)-$,
- 25 c) $-C(O)NR^{10}-$,
- d) $-NR^{10}C(O)-$,
- e) $-NR^{10}C(O)-O-$,
- f) $-O-C(O)NR^{10}-$,
- g) $-NR^{10}C(O)NR^{10}-$,
- 30 h) O,
- i) $-N(R^{10})-$,
- j) $-S(O)_2N(R^{10})-$,
- k) $-N(R^{10})S(O)_2-$, or
- l) $S(O)_m$;

Y is selected from

- a) a bond,
- b) $-C(=O)-$,
- 5 c) $-C(O)NR^{10}-$,
- d) $-C(O)O-$, or
- e) $-S(=O)_m$;

Z^1 is selected from unsubstituted or substituted aryl and unsubstituted or substituted
 10 heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with
 one or more of:

- 1) C_{1-8} alkyl, C_{2-8} alkenyl or C_{2-8} alkynyl, unsubstituted or substituted
 with:
 - a) C_{1-4} alkoxy,
 - 15 b) NR^6R^7 ,
 - c) C_{3-6} cycloalkyl,
 - d) aryl or heterocycle,
 - e) HO,
 - f) $-S(O)_mR^4$,
 - 20 g) $-C(O)NR^6R^7$, or
 - h) C_{1-4} perfluoroalkyl;
- 2) substituted or unsubstituted aryl or substituted or unsubstituted
 heterocycle,
- 3) halogen,
- 25 4) OR^6 ,
- 5) NR^6R^7 ,
- 6) CN,
- 7) NO_2 ,
- 8) CF_3 ;
- 30 9) $-S(O)_mR^4$,
- 10) $-OS(O)_2R^4$,
- 11) $-C(O)NR^6R^7$,
- 12) $-C(O)OR^6$, or
- 13) C_3-C_6 cycloalkyl;

35

Z^2 is selected from a bond, unsubstituted or substituted aryl and unsubstituted or substituted heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with one or more of:

- 1) C_{1-8} alkyl, C_{2-8} alkenyl or C_{2-8} alkynyl, unsubstituted or substituted
 5 with:
 - a) C_{1-4} alkoxy,
 - b) NR^6R^7 ,
 - c) C_{3-6} cycloalkyl,
 - d) aryl or heterocycle,
 - 10 e) HO,
 - f) $-S(O)_mR^4$,
 - g) $-C(O)NR^6R^7$, or
 - h) C_{1-4} perfluoroalkyl;
- 2) substituted or unsubstituted aryl or substituted or unsubstituted
 15 heterocycle,
- 3) halogen,
- 4) OR^6 ,
- 5) NR^6R^7 ,
- 6) CN,
- 20 7) NO_2 ,
- 8) CF_3 ,
- 9) $-S(O)_mR^4$,
- 10) $-OS(O)_2R^4$,
- 11) $-C(O)NR^6R^7$,
- 25 12) $-C(O)OR^6$, or
- 13) C_3-C_6 cycloalkyl;

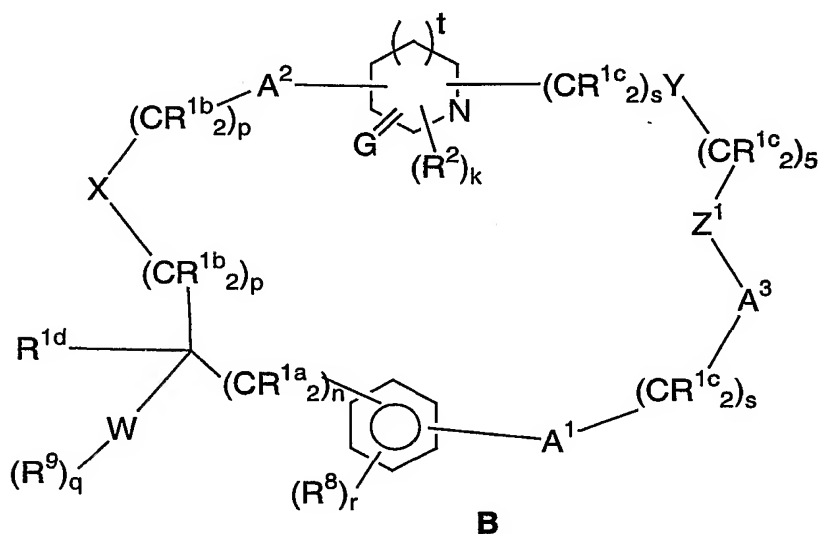
- | | |
|--------------------|---------------------|
| k is | 0, 1, 2, 3 or 4; |
| m is | 0, 1 or 2; |
| 30 n is | 0, 1, 2, 3, 4 or 5; |
| p is independently | 0, 1, 2, 3, 4 or 5; |
| q is | 1, 2 or 3; |
| r is | 0, 1, 2, 3, 4 or 5; |
| s is independently | 0, 1, 2, 3, 4 or 5; |

t is 0 or 1; and
u is independently 2, 3, 4, 5 or 6;

or a pharmaceutically acceptable salt or stereoisomer thereof.

5

3. The compound, according to Claim 1, of formula B:



wherein:

10

R^{1a}, R^{1b} and R^{1c} are independently selected from:

- 15
- a) hydrogen,
 - b) unsubstituted or substituted aryl,
 - c) unsubstituted or substituted heterocycle,
 - d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 - e) -N(R¹⁰)₂,
 - f) unsubstituted or substituted C₂-C₆ alkenyl
 - g) -OR¹⁰, or
 - h) unsubstituted or substituted C₁-C₆ alkyl,

20

wherein the substituent on the substituted group is selected from unsubstituted or substituted aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, R¹⁰O-, and -N(R¹⁰)₂,

or two R^{1a}s, two R^{1b}s or two R^{1c}s, on the same carbon atom may be combined to form -(CH₂)_u-;

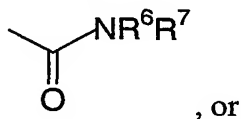
R^{1d} is selected from:

- 5 a) hydrogen,
- b) unsubstituted or substituted aryl,
- c) unsubstituted or substituted heterocycle,
- d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- e) R¹⁰O-,
- 10 f) R¹¹S(O)_m-,
- g) R¹⁰C(O)NR¹⁰-,
- h) (R¹⁰)₂NC(O)-,
- i) CN,
- j) NO₂,
- 15 k) R¹⁰C(O)-,
- l) R¹⁰OC(O)-,
- m) -N(R¹⁰)₂,
- n) R¹¹OC(O)NR¹⁰-,
- o) unsubstituted or substituted C₁-C₆ alkyl,
- 20 p) unsubstituted or substituted C₂-C₆ alkenyl, or
- q) unsubstituted or substituted C₂-C₆ alkynyl,

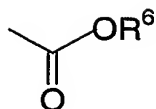
25 wherein the substituent on the substituted group is selected from unsubstituted or substituted aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, and R¹¹OC(O)NR¹⁰-;

R² is independently selected from

- 30 a) hydrogen,
- b) unsubstituted or substituted C₁₋₈ alkyl,
- c)



d)

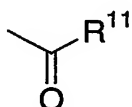


wherein the substituted group is substituted with one or more of:

- 1) aryl or heterocycle, unsubstituted or substituted with:
 - a) C₁₋₄ alkyl,
 - b) (CH₂)_pOR⁶,
 - c) (CH₂)_pNR⁶R⁷,
 - d) halogen,
 - e) CN,
- 2) OR⁶,
- 3) SR⁴, S(O)R⁴, SO₂R⁴, or
- 4) -NR⁶R⁷;

R⁴ is selected from C₁₋₄ alkyl, or C₃₋₆ cycloalkyl, unsubstituted or substituted with:

- 1) C₁₋₄ alkoxy,
- 2) aryl or heterocycle,
- 3) halogen,
- 4) HO,
- 5)



20

- 6) SO₂R¹¹,
- 7) N(R¹⁰)₂, or
- 8) C₁₋₄ perfluoroalkyl;

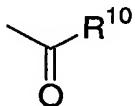
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R⁶ and R⁷ are independently selected from:

- a) hydrogen,
- b) R¹⁰C(O)-,
- c) R¹⁰OC(O)-, or
- d) C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₆ cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl,

30

unsubstituted or substituted with one or more substituents selected from:

- 5
- 1) $R^{10}O-$,
 - 2) aryl or heterocycle,
 - 3) halogen,
 - 4) $R^{10}C(O)NR^{10}-$,
 - 5) 
- 10
- 6) SO_2R^{11} ,
 - 7) $N(R^{10})_2$,
 - 8) C₃₋₆ cycloalkyl,
 - 9) C₁₋₆ perfluoroalkyl,
 - 10) $R^{10}OC(O)-$,
 - 15 11) $R^{11}OC(O)NR^{10}-$,
 - 12) CN, and
 - 13) NO₂; or

R⁶ and R⁷ may be joined in a ring;

20

R⁸ is independently selected from:

- 25
- a) hydrogen,
 - b) unsubstituted or substituted aryl,
 - c) unsubstituted or substituted heterocycle,
 - d) unsubstituted or substituted C₃₋₁₀ cycloalkyl,
 - e) unsubstituted or substituted C₂₋₆ alkenyl,
 - f) unsubstituted or substituted C₂₋₆ alkynyl,
 - g) C₁₋₆ perfluoroalkyl,
 - h) halo,
 - 30 i) $R^{10}O-$,
 - j) $R^{11}S(O)_m-$,
 - k) $R^{10}C(O)NR^{10}-$,
 - l) $(R^{10})_2NC(O)-$,

- 5 m) CN,
 n) NO₂,
 o) R¹⁰C(O)-,
 p) R¹⁰OC(O)-,
 q) -N(R¹⁰)₂,
 r) R¹¹OC(O)NR¹⁰-, or
 s) unsubstituted or substituted C₁-C₆ alkyl, optionally substituted
 by unsubstituted or substituted aryl, unsubstituted or substituted
 heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl,
 10 C₁-C₆ perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)
 NR¹⁰-, (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or
 R¹⁰OC(O) NR¹⁰-;

R⁹ is selected from:

- 15 a) hydrogen,
 b) C₁-C₆ perfluoroalkyl,
 c) R¹⁰O-,
 d) R¹⁰C(O)NR¹⁰-,
 e) (R¹⁰)₂NC(O)-, or
 20 f) unsubstituted or substituted C₁-C₆ alkyl optionally substituted by
 C₁-C₆ perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)
 NR¹⁰-, (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or
 R¹¹OC(O)NR¹⁰-;

25 R¹⁰ is independently selected from

- a) hydrogen,
 b) unsubstituted or substituted C₁-C₆ alkyl,
 c) unsubstituted or substituted C₁-C₃ perfluoroalkyl,
 d) unsubstituted or substituted benzyl,
 30 e) unsubstituted or substituted aryl, or
 f) unsubstituted or substituted heterocycle;

R¹¹ is independently selected from

- a) unsubstituted or substituted C₁-C₆ alkyl,
 35 b) unsubstituted or substituted aryl, or

c) unsubstituted or substituted heterocycle;

A¹ and A³ are independently selected from

- 5 a) a bond,
 b) -C(O)-,
 c) -C(O)NR¹⁰-,
 d) -NR¹⁰C(O)-,
 e) O,
 f) -N(R¹⁰)-,
10 g) -S(O)₂N(R¹⁰)-,
 h) -N(R¹⁰)S(O)₂-, or
 i) S(O)_m;

A² is selected from

- 15 a) a bond,
 b) -C(O)-,
 c) -C(O)NR¹⁰-,
 d) -NR¹⁰C(O)-,
 e) O,
20 f) -N(R¹⁰)-,
 g) -S(O)₂N(R¹⁰)-,
 h) -N(R¹⁰)S(O)₂-,
 i) -NR¹⁰C(O)NR¹⁰-, or
 j) S(O)_m;

25

G is selected from H₂ or O;

W is a heterocycle selected from imidazolyl, pyridyl, or triazolyl;

30 X is selected from:

- a) a bond,
 b) -C(O)-,
 c) -C(O)NR¹⁰-,
 d) -NR¹⁰C(O)-,

- 5
- e) $-\text{NR}^{10}\text{C}(\text{O})-\text{O}-$,
 - f) $-\text{O}-\text{C}(\text{O})\text{NR}^{10}-$,
 - g) $-\text{NR}^{10}\text{C}(\text{O})\text{NR}^{10}-$,
 - h) O ,
 - i) $-\text{N}(\text{R}^{10})-$,
 - j) $-\text{S}(\text{O})_2\text{N}(\text{R}^{10})-$,
 - k) $-\text{N}(\text{R}^{10})\text{S}(\text{O})_2-$, or
 - l) $\text{S}(\text{O})_m$;

10 Y is selected from

- a) a bond,
- b) $-\text{C}(=\text{O})-$,
- c) $-\text{C}(\text{O})\text{NR}^{10}-$,
- d) $-\text{C}(\text{O})\text{O}-$, or
- 15 e) $-\text{S}(=\text{O})_m$;

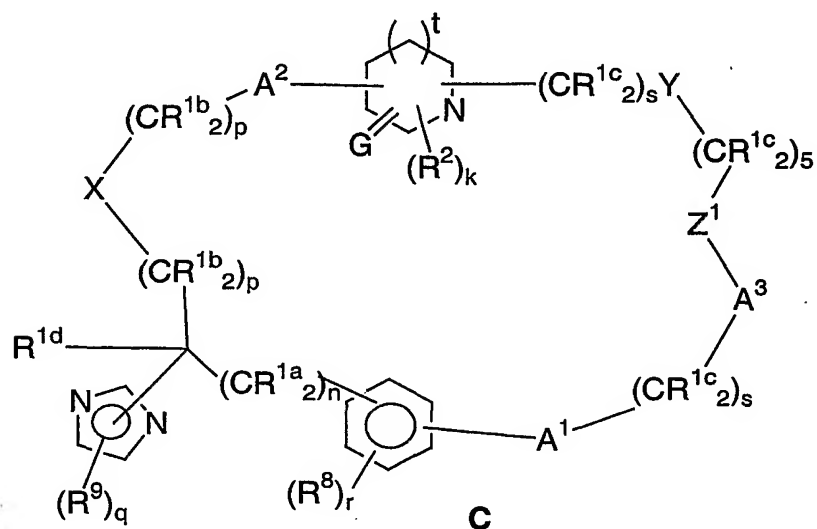
Z^1 is selected from unsubstituted or substituted aryl and unsubstituted or substituted heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with one or more of:

- 20 1) C_{1-8} alkyl, C_{2-8} alkenyl or C_{2-8} alkynyl, unsubstituted or substituted with:
- a) C_{1-4} alkoxy,
 - b) NR^6R^7 ,
 - c) C_{3-6} cycloalkyl,
 - 25 d) aryl or heterocycle,
 - e) HO ,
 - f) $-\text{S}(\text{O})_m\text{R}^4$,
 - g) $-\text{C}(\text{O})\text{NR}^6\text{R}^7$, or
 - h) C_{1-4} perfluoroalkyl;
- 30 2) substituted or unsubstituted aryl or substituted or unsubstituted heterocycle,
- 3) halogen,
 - 4) OR^6 ,
 - 5) NR^6R^7 ,

- 6) CN,
 7) NO₂,
 8) CF₃;
 9) -S(O)_mR⁴,
 10) -OS(O)₂R⁴,
 11) -C(O)NR⁶R⁷,
 12) -C(O)OR⁶, or
 13) C₃-C₆ cycloalkyl;
- 10 k is 0, 1, 2, 3 or 4;
 m is 0, 1 or 2;
 n is 0, 1, 2, 3, 4 or 5;
 p is independently 0, 1, 2, 3, 4 or 5;
 q is 1, 2 or 3;
 15 r is 0, 1, 2, 3, 4 or 5;
 s is independently 0, 1, 2, 3, 4 or 5;
 t is 0 or 1; and
 u is independently 2, 3, 4, 5 or 6;

20 or a pharmaceutically acceptable salt or stereoisomer thereof.

4. The compound, according to Claim 1, of formula C:



wherein:

R^{1a}, R^{1b} and R^{1c} are independently selected from:

- a) hydrogen,
 - 5 b) unsubstituted or substituted aryl,
 - c) unsubstituted or substituted heterocycle,
 - d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 - e) -N(R¹⁰)₂,
 - f) unsubstituted or substituted C₂-C₆ alkenyl
 - 10 g) -OR¹⁰, or
 - h) unsubstituted or substituted C₁-C₆ alkyl,
- wherein the substituent on the substituted group is selected from unsubstituted or substituted aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, R¹⁰O-, and -N(R¹⁰)₂,
- 15 or two R^{1a}s, two R^{1b}s or two R^{1c}s, on the same carbon atom may be combined to form -(CH₂)_u-;

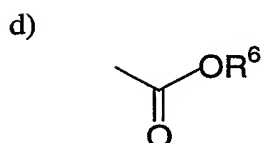
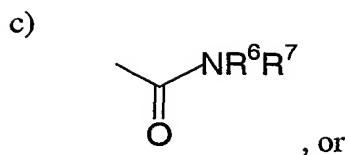
R^{1d} is selected from:

- a) hydrogen,
 - 20 b) unsubstituted or substituted aryl,
 - c) unsubstituted or substituted heterocycle,
 - d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 - e) R¹⁰O-,
 - f) -N(R¹⁰)₂, or
 - 25 g) unsubstituted or substituted C₁-C₆ alkyl,
- wherein the substituent on the substituted group is selected from unsubstituted or substituted aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, R¹⁰O-, R¹⁰C(O)NR¹⁰-, (R¹⁰)₂NC(O)-, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, and R¹¹OC(O)-NR¹⁰-;

30

R² is independently selected from

- a) hydrogen,
- b) unsubstituted or substituted C₁₋₈ alkyl,



5 wherein the substituted group is substituted with one or more of:

- 1) aryl or heterocycle, unsubstituted or substituted with:
 - a) C₁₋₄ alkyl,
 - b) (CH₂)_pOR⁶,
 - c) (CH₂)_pNR⁶R⁷,
 - 10 d) halogen,
 - e) CN,
- 2) OR⁶,
- 3) SR⁴, S(O)R⁴, SO₂R⁴, or
- 4) -NR⁶R⁷;

15

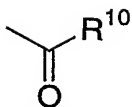
R⁴ is selected from C₁₋₄ alkyl, or C₃₋₆ cycloalkyl, unsubstituted or substituted with:

- 1) C₁₋₄ alkoxy,
- 2) aryl or heterocycle,
- 3) halogen, or
- 20 4) -OH;

R⁶ and R⁷ are independently selected from:

- a) hydrogen, or
- b) C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ perfluoroalkyl,
- 25 C₃₋₆ cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with one or more substituents selected from:
 - 1) R¹⁰O-,
 - 2) aryl or heterocycle,
 - 30 3) halogen,
 - 4) R¹⁰C(O)NR¹⁰-,

5)

6) SO_2R^{11} ,7) $\text{N}(\text{R}^{10})_2$, or5 8) C_{3-6} cycloalkyl, or

R^6 and R^7 may be joined in a ring;

R^8 is independently selected from:

- 10 a) hydrogen,
 b) unsubstituted or substituted aryl,
 c) unsubstituted or substituted heterocycle,
 d) unsubstituted or substituted $\text{C}_3\text{-C}_{10}$ cycloalkyl,
 e) unsubstituted or substituted $\text{C}_2\text{-C}_6$ alkenyl,
 15 f) unsubstituted or substituted $\text{C}_2\text{-C}_6$ alkynyl,
 g) $\text{C}_1\text{-C}_6$ perfluoroalkyl,
 h) halo,
 i) $\text{R}^{10}\text{O}-$,
 j) $\text{R}^{11}\text{S}(\text{O})_m-$,
 20 k) $\text{R}^{10}\text{C}(\text{O})\text{NR}^{10}-$,
 l) $(\text{R}^{10})_2\text{NC}(\text{O})-$,
 m) CN ,
 n) NO_2 ,
 o) $\text{R}^{10}\text{C}(\text{O})-$,
 25 p) $\text{R}^{10}\text{OC}(\text{O})-$,
 q) $-\text{N}(\text{R}^{10})_2$,
 r) $\text{R}^{11}\text{OC}(\text{O})\text{NR}^{10}-$, and
 s) unsubstituted or substituted $\text{C}_1\text{-C}_6$ alkyl, optionally substituted
 by unsubstituted or substituted aryl, unsubstituted or substituted
 30 heterocycle, $\text{C}_3\text{-C}_{10}$ cycloalkyl, $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl,
 $\text{C}_1\text{-C}_6$ perfluoroalkyl, F, Cl, Br, $\text{R}^{10}\text{O}-$, $\text{R}^{11}\text{S}(\text{O})_m-$, $\text{R}^{10}\text{C}(\text{O})\text{NR}^{10}-$,
 $(\text{R}^{10})_2\text{NC}(\text{O})-$, CN , $\text{R}^{10}\text{C}(\text{O})-$, $\text{R}^{10}\text{OC}(\text{O})-$, $-\text{N}(\text{R}^{10})_2$, or
 $\text{R}^{10}\text{OC}(\text{O})\text{NR}^{10}-$;

R⁹ is selected from:

- a) hydrogen,
- b) C₁-C₆ perfluoroalkyl, or
- 5 c) unsubstituted or substituted C₁-C₆ alkyl optionally substituted by C₁-C₆ perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, (R¹⁰)₂NC(O)-, CN, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-;

10 R¹⁰ is independently selected from

- a) hydrogen,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₁-C₃ perfluoroalkyl,
- d) unsubstituted or substituted benzyl,
- 15 e) unsubstituted or substituted aryl, or
- f) unsubstituted or substituted heterocycle;

R¹¹ is independently selected from

- a) unsubstituted or substituted C₁-C₆ alkyl,
- 20 b) unsubstituted or substituted aryl, or
- c) unsubstituted or substituted heterocycle;

A¹ and A³ are independently selected from

- a) a bond,
- 25 b) -C(O)-,
- c) -C(O)NR¹⁰-,
- d) -NR¹⁰C(O)-,
- e) O,
- f) -N(R¹⁰)-,
- 30 g) -S(O)₂N(R¹⁰)-,
- h) -N(R¹⁰)S(O)₂-, or
- i) S(O)_m;

A² is selected from

- 35 a) a bond,

- b) $-C(O)-$,
 c) $-C(O)NR^{10}-$,
 d) $-NR^{10}C(O)-$,
 e) O,
 5 f) $-N(R^{10})-$,
 g) $-S(O)_2N(R^{10})-$,
 h) $-N(R^{10})S(O)_2-$,
 i) $-NR^{10}C(O)NR^{10}-$, or
 j) $S(O)_m$;

10

G is selected from H_2 or O;

X is selected from:

- a) a bond,
 15 b) $-C(O)-$,
 c) $-C(O)NR^{10}-$,
 d) $-NR^{10}C(O)-$,
 e) $-NR^{10}C(O)-O-$,
 f) $-O-C(O)NR^{10}-$,
 20 g) $-NR^{10}C(O)NR^{10}-$,
 h) O,
 i) $-N(R^{10})-$,
 j) $-S(O)_2N(R^{10})-$,
 k) $-N(R^{10})S(O)_2-$, or
 25 l) $S(O)_m$;

Y is selected from

- a) a bond,
 b) $-C(=O)-$,
 30 c) $-C(O)NR^{10}-$,
 d) $-C(O)O-$, or
 e) $-S(=O)_m$;

35 Z^1 is selected from unsubstituted or substituted aryl and unsubstituted or substituted heterocycle, wherein the substituted aryl or substituted heterocycle is substituted with

one or more of:

- 1) C₁₋₈ alkyl, C₂₋₈ alkenyl or C₂₋₈ alkynyl, unsubstituted or substituted with:
 - a) C₁₋₄ alkoxy,
 - 5 b) NR⁶R⁷,
 - c) C₃₋₆ cycloalkyl,
 - d) aryl or heterocycle,
 - e) HO,
 - f) -S(O)_mR⁴,
 - 10 g) -C(O)NR⁶R⁷, or
 - h) C₁₋₄ perfluoroalkyl;
- 2) substituted or unsubstituted aryl or substituted or unsubstituted heterocycle,
- 3) halogen,
- 15 4) OR⁶,
- 5) NR⁶R⁷,
- 6) CN,
- 7) NO₂,
- 8) CF₃;
- 20 9) -S(O)_mR⁴,
- 10) -OS(O)₂R⁴,
- 11) -C(O)NR⁶R⁷,
- 12) -C(O)OR⁶, or
- 13) C_{3-C6} cycloalkyl;

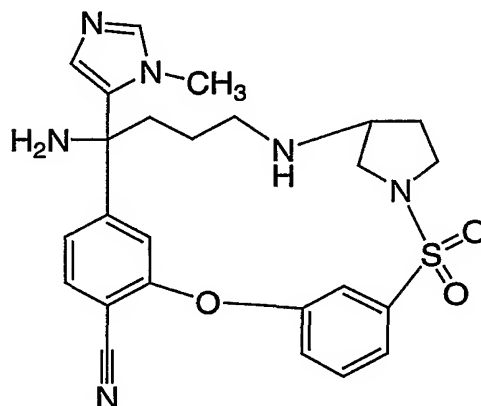
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k is 0, 1, 2, 3 or 4;
 m is 0, 1 or 2;
 n is 0, 1, 2, 3, 4 or 5;
 p is independently 0, 1, 2, 3, 4 or 5;
 30 q is 1, 2 or 3;
 r is 0, 1, 2, 3, 4 or 5;
 s is independently 0, 1, 2, 3, 4 or 5;
 t is 0 or 1; and
 u is independently 2, 3, 4, 5 or 6;

35

or a pharmaceutically acceptable salt or stereoisomer thereof.

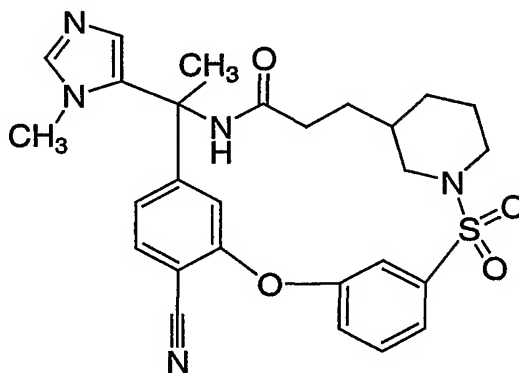
5. A compound which is selected from:



5

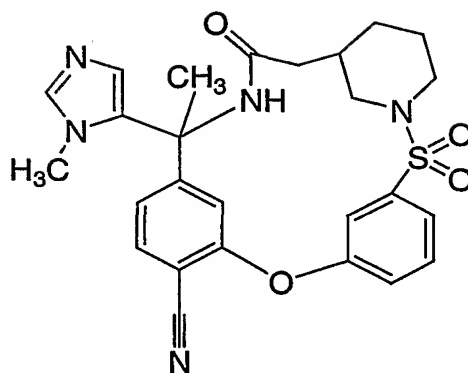
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10

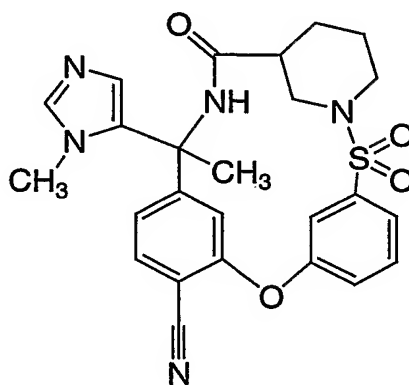


(14*R or S*,19*S or R*)-14-methyl-14-(1-methyl-1H-imidazol-5-yl)-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[17.3.1.1^{3,7}.1^{9,13}]pentacos-3(25),4,6,9(24),10,12-hexaene-10-carbonitrile 2,2-dioxide

15



(14*R* or *S*, 18*R* or *S*) -14-methyl-14-(1-methyl-1*H*-imidazol-5-yl)-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[16.3.1.1^{3,7}.1^{9,13}]tetracos-3(24),4,6,9(23),10,12-hexaene-10-carbonitrile 2,2-dioxide



(14*R* or *S*, 17*R* or *S*) -14-methyl-14-(1-methyl-1*H*-imidazol-5-yl)-16-oxo-8-oxa-2-thia-1,15-diazatetracyclo[15.3.1.1^{3,7}.1^{9,13}]tricos-3(23),4,6,9(22),10,12-hexaene-10-carbonitrile 2,2-dioxide

or a pharmaceutically acceptable salt or stereoisomer thereof.

6. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 1.

7. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 4.
- 5 8. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 5.
- 10 9. A method for inhibiting prenyl-protein transferase which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.
- 15 10. A method for inhibiting prenyl-protein transferase which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 4.
- 20 11. A method for inhibiting prenyl-protein transferase which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 5.
- 25 12. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.
- 30 13. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 4.
- 35 14. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 5.
15. A method for treating neurofibromin benign proliferative disorder which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

16. A method for treating blindness related to retinal vascularization which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.
- 5
17. A method for treating infections from hepatitis delta and related viruses which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.
- 10
18. A method for preventing restenosis which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.
- 15
19. A method for treating polycystic kidney disease which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.
- 20
20. A method of conferring radiation sensitivity on a tumor cell using a therapeutically effective amount of a compound of Claim 1 in combination with radiation therapy.
21. A method of using a therapeutically effective amount of a compound of Claim 1 in combination with an antineoplastic to treat cancer.
- 25
22. A method according to Claim 21 wherein the antineoplastic is paclitaxel.
23. A pharmaceutical composition made by combining the compound of Claim 1 and a pharmaceutically acceptable carrier.
- 30
24. A process for making a pharmaceutical composition comprising combining a compound of Claim 1 and a pharmaceutically acceptable carrier.

SEQUENCE LISTING

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deSolms, S. Jane
Shaw, Anthony W.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/27013

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/44, 31/415; C07D 291/00
US CL : 514/286, 397; 540/456, 469

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/286, 397; 540/456, 469

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST (540/456, 469), CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,E	US 6,316,436 B1 (DESOLMS et al.) 13 November 2001.	1-24

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search

10 December 2001 (10.12.2001)

Date of mailing of the international search report

16 JAN 2002

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

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